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BIOCHEMICAL STUDIES ON THE NITROGEN COMPOUNDS OF ALGAE

Chinkichi Ogino

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INTRODUCTION

Although remarkable progress has recently been made concerning the nitrogen metabolism in higher plants as well as in certain microbes such as bacteria, yeasts and molds, relatively little is known about the biochemistry of nitrogen compounds in algae. The main process of the nitrogen metabolism between higher plants and algae seems not so greatly different each other. However, following respects should be taken into consideration: (1) the stage in the progress of the evolution is different in both plants, (2) the algae include many classes of plants which are phylogenetically different, (3) in the organization of the plant, there exist several kinds of the type from unicellular to multicellular body. Considering these points, some differences in the nitrogen metabolism may be expected not only between algae and higher plants but among different species of algae.

From these points of view, main efforts of the present studies have been dedicated to the elucidation of the nature of nitrogen compounds which seemed essential to algae. As it is difficult in this investigation to cover entire groups of the algae, marine algae belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae including some fresh water ones were used. In organisms nitrogen exists for the most part in the form of protein and besides this a small quantity of non-protein nitrogen compounds exists. As these nitrogen compounds are biochemically closely related one another, it seems necessary to make clear the nature of each non-protein nitrogen compound in order to disclose the mechanism of the nitrogen metabolism. Hence, the special attention has been paid in the present study to reveal the nature of non-protein nitrogen compounds in algae and the change of their contents.

Naturally it may be considered probable that the content of each nitrogen compound varies with age, habitat conditions, and growing season of the algae. Besides these the amount of various nitrogen compounds of the algae was found to fluctuate more or less significantly among individuals of the same age and habitat. Accordingly in the present research samples for analysis were taken from as many individuals as possible of each species from the same habitat.

This investigation deals first with the analysis of several forms of nitrogen compounds of algae, i.e., total N, protein N, and non-protein N with particular reference to their change with the part and age of the frond as well as with the season of the growth. Secondly the study was made for the elucidation of the nature of the non-protein nitrogen compounds, of which the greater part are free amino acids and the smaller one polypeptides and various bases.

The author wishes to express his sincere gratitude to Dr. Tomoo MIWA, Professor of the Tokyo University of Education, for his kind supervision throughout the course of the present study. The author is also indebted to the late Dr. Takeo OYA for his invaluable advice and encouragement. He is also under obligation to Drs. S. UEDA, M. MIGITA, S. INO, H. HIRANO and Mr. K. SAKUMA for their kindness shown in various ways. This study was supported in part by a Grant in Aid for Developmental Scientific Research from the Ministry of Edu-

cation to which the author offers his hearty thanks.

MATERIALS

Samples of the marine algae used in the present study were taken from the coast of Tokyo Bay, Sagami Bay as well as the Pacific coast of Japan, and those of fresh water algae, *Prasiola japonica*, at Hikawa, upper stream of the Tama River, and *Cladophora* sp., at Yokosuka. Samples were dried in the air as fast as possible after the collection, further dried in a desiccator or in an electric oven at 80°C and ground to powder. The content of non-protein N suffered no change by these treatments, since no appreciable difference of the non-protein N content could be observed between the samples treated as above and the those which were preserved in alcohol immediately after the collection (see Nos. 15 and 16 of Table 1).

CHAPTER I

TOTAL-, PROTEIN-, AND NON-PROTEIN-N CONTENTS OF ALGAE AND THEIR SEASONAL VARIATION

Numerous investigations have already been reported on the content of crude protein of algae until now[1)-3)]. However, very little is known as to the separate determination of the total-, protein-, and non-protein-N contents. Only Fujikawa[4)] has reported about these problems with *Porphyra* and *Enteromorpha*.

The writer has conducted the estimation of these nitrogen fractions with a number of marine algae belonging to Chlorophyceae, Phaeophyceae, and Rhodophyceae including some fresh water algae. Attention was paid to the variation of their contents with the age, season of the year and with the part of the frond. The ratio of non-protein N to total N was calculated. The results are shown in Tables 1 to 3 and Figs. 1 to 3.

Method of Determination

Nitrogen determination was made by the Kjeldahl method. Non-protein N was determined by the following procedure. Samples varying in weight from 1 to 5 g were extracted three times with water (300–500 ml. in each) on a boiling water bath each for 30 minutes, and filtered through a cotton wool. The filtrates and washings were combined and purified with lead acetate. The excess of lead in the filtrate was precipitated with sulfuric acid and filtered again. The filtrates and washings were combined, neutralized to pH 5 with dilute sodium hydroxide, concentrated on a boiling water bath, and made up to 100 ml. The content of non-protein N was determined with the aliquot of this solution. The protein N content was calculated by subtracting the non-protein N from the total N. When the filtration of the lead acetate precipitate was difficult lead subacetate was used in its place. In some samples of red algae further addition of a small amount of baryta water was necessary. The values of protein N of Nos. 13–17 of Table 2 were obtained by the Stutzer's method.

Results and Discussion

As shown in the tables, the content of total-, protein-, and non-protein-N of algae vary not only with the species, season of the growth, part of the frond but also with the age in the same species. On the whole, however, the content of total N is relatively high in green algae especially in the species belonging to Ulotrichales and Siphonocladiales and low in brown algae with some exceptions. In red algae, considerable difference in the total N content can be seen between Bangiales and Florideae, namely those of Bangiales are high and those of Florideae low. *Porphyra tenera* belonging to Bangiales is the highest in total N content thus far observed. As to total N contents in several other species of Bangiales similar results have been reported by TAKAGI[5]. Accordingly such a trend may be considered as one of the important properties of Bangiales. As mentioned above, the content of total N varies with the age and the part of the frond in the same species. Thus, in *Undaria pinnatifida*, the sporophyll shows higher value compared with the vegetative frond. In *Higikia fusiformis*, the total N content of the old alga is higher than that of the young one; in *Gloiopeltis furcata*, that of the young alga is higher than that of the old one.

The content of protein N of algae shows nearly the same tendency as the total N content.

Table 1. Total-, Protein-, and Non-Protein-N Contents of Green Algae in Per Cent on Dry Basis. *, fresh water algae.

Name of Algae	Total N	Protein N	Non-Protein N	$\frac{\text{Non-Protein N}}{\text{Total N}} \times 100$	Locality of Collection	Date of Collection
Ulotrichales:						
1) *Ulva pertusa*	2.68	2.49	0.19	7.1	Chiba	—
2) 〃 〃	5.07	3.80	1.26	21.1	Tokyo	Nov. 25
3) *Ulva conglobata*	2.09	—	—	—	Chiba	May 12
4) *Enteromorpha Linza*	5.72	4.90	0.82	14.3	Tokyo	Feb. 20
5) *E. intestinalis*	1.28	1.14	0.14	11.1	Kominato	May 16
6) *E. compressa*	4.43	3.64	0.79	17.8	Enoshima	Feb. 8
7) *Prasiala japonica**	5.28	4.12	1.16	22.1	Hikawa	Jul. 8
8) 〃 〃	5.63	4.03	1.60	28.3	〃	Sept. 23
9) 〃 〃	6.25	4.28	1.97	31.6	〃	Nov. 3
10) 〃 〃	6.36	4.16	2.20	34.5	〃	Dec. 29
11) 〃 〃	5.87	4.23	1.64	28.0	〃	Feb. 9
12) 〃 〃	5.50	3.25	2.25	40.8	〃	Mar. 11
13) 〃 〃	5.84	3.92	1.92	33.0	Nippara	Dec. 30
14) 〃 〃	5.83	4.19	1.64	28.1	〃	Feb. 6
15) 〃 〃	4.64	3.63	1.01	21.6	Hikawa	Aug. 21
16) 〃 〃	—	—	0.99	—	〃	Aug. 21
Siphonocladiales:						
17) *Cladophora Wrightiana*	5.76	5.16	0.61	10.5	Tokyo	Nov. 25
18) *Cladophora* sp.*	5.48	4.76	0.73	13.2	Yokosuka	Sept. 1
19) 〃 〃	5.13	4.71	0.42	8.2	〃	May 8
Siphonales:						
20) *Codium latum*	2.60	—	—	—	Tokyo	Nov. 25
21) *Codium fragile*	1.71	—	—	—	〃	Nov. 25

The content of non-protein N varies, in general, with different algae. These differences are also observed according to the part of the frond and with the age in the same species. In *Undaria pinnatifida*, the sporophyll shows higher value than the vegetative frond; in both *Higikia fusiformis* and *Gloiopertis furcata* the young alga shows higher value compared with the old one. *Prasiola japonica*, a fresh water green algae, shows considerably high value in the non-protein N content, being over 2 per cent in winter on dry basis.

The ratios of non-protein N to total N vary with different algae. Some species of brown algae, i.e., *Laminaria* sp., *Undaria pinnatifida*, and *Sargassum Ringgoldianum* show high values of this ratio and those of red algae of both Bangiales and Florideae show but little fluctuation. In green algae the ratios are about the same as those of red algae, except *Prasiola*. *Prasiola japonica* shows relatively high value of this ratio, especially in winter. The ratio non-protein N/total N varies also with the age and with the part of the frond in the same species. Namely, the young frond of *Gloiopeltis furcata* shows

Table 2. Total-, Protein-, and Non-Protein-N Contents of Brown Algae in Per Cent on Dry Basis.

Name of Algae	Total N	Protein N	Non-Protein N	$\frac{\text{Non-Protein N}}{\text{Total N}} \times 100$	Locality of Collection	Date of Collection
Laminarineae:						
1) *Laminaria* sp.	1.68	1.00	0.68	40.7	—	—
2) 〃 〃	1.43	1.27	0.16	11.2	—	—
3) *Eisenia bicyclis*	2.27	1.80	0.47	20.8	Yokosuka	Apr. 25
4) *Ecklonia cava*	1.63	1.42	0.21	12.6	〃	May 5
5) *Undaria pinnatifida* (leaf)	1.79	1.33	0.46	25.9	〃	Apr. 9
6) 〃 〃 (sporophyll)	2.23	1.57	0.66	29.6	〃	Apr. 9
7) 〃 〃 (stem)	1.55	1.09	0.46	29.5	〃	Apr. 9
Fucineae:						
8) *Sargassum Ringgoldianum*	2.20	1.54	0.66	30.0	〃	May 9
9) 〃 〃	2.29	1.38	0.91	39.7	Chiba	—
10) 〃 *hemiphyllum*	2.39	2.00	0.39	16.1	Yokosuka	May 10
11) 〃 *Thunbergii*	2.11	2.01	0.10	4.8	Kominato	May 16
12) *Cystophyllum sisymbrioides*	1.83	1.57	0.26	14.0	Yokosuka	May 10
13) *Higikia fusiformis*	1.68	1.39	0.29	17.2	Kominato	Jan. 17
14) 〃 〃	1.79	1.60	0.19	10.7	〃	Feb. 24
15) 〃 〃	1.59	1.32	0.27	17.1	〃	Mar. 30
16) 〃 〃	0.92	0.91	0.01	1.0	〃	Jul. 4
17) 〃 〃 (young frond)	0.86	0.70	0.16	18.9	〃	Jul. 4
18) *Ishige Okamurai*	1.96	1.68	0.28	14.4	〃	May 16
Ectocarpineae:						
19) *Myelophycus caespitosus*	2.09	—	—	—	Chiba	May 12
Dictyotineae:						
20) *Padina arborescens*	1.52	—	—	—	Chiba	May 12

Table 3. Total-, Protein-, and Non-Protein-N Contents of Red Algae in Per Cent on Dry Basis.

Name of Algae	Total N	Protein N	Non-Protein N	$\frac{\text{Non-Protein N}}{\text{Total N}} \times 100$	Locality of Collection	Date of Collection
Bangiales:						
1) *Porphyra tenera*	6.49	5.67	0.82	12.7	Chiba	Nov. 15, 1949
2) 〃 〃	7.79	6.71	1.08	13.8	〃	Dec. 8, 1949
3) 〃 〃	6.11	5.13	0.98	15.9	〃	Dec. 28, 1949
4) 〃 〃	6.33	5.46	0.87	13.7	〃	Jan. 18, 1950
5) 〃 〃	5.99	5.18	0.81	13.6	〃	Feb. 5, 1950
6) 〃 〃	4.20	3.54	0.66	15.6	〃	Mar. 2, 1950
7) 〃 〃	6.66	5.80	0.86	12.8	〃	Jan. 13, 1951
8) 〃 〃	6.19	5.35	0.84	13.6	〃	Jan. 26, 1951
9) 〃 〃	6.15	5.40	0.75	12.2	〃	Mar. 2, 1951
Florideae:						
10) *Gelidium elegans*	1.68	1.46	0.22	13.1	Wakayama	—
11) *Gloiopeltis furcata* (young frond)	2.51	2.00	0.51	20.4	Kominato	May 16
12) 〃 〃 (old frond)	1.70	1.45	0.25	14.7	〃	〃
13) 〃 〃 (sporophyll)	2.50	2.06	0.44	17.6	〃	〃
14) 〃 *cervicornis*	2.45	2.11	0.34	13.8	〃	〃
15) *Grateloupia filicina*	2.07	1.78	0.29	13.8	〃	〃
16) 〃 *elliptica*	2.09	1.97	0.12	5.7	Yokosuka	May 8
17) 〃 *ligulata*	2.33	—	—	—	Enoshima	May 29
18) *Corallina officinalis*	0.64	0.53	0.10	16.1	Kominato	May 16
19) *Chondrus ocellatus*	2.77	2.45	0.32	11.4	Yokosuka	May 9
20) 〃 〃 *f. canaliculatus*	1.78	—	—	—	Enoshima	May 29
21) *Gigartina intermedia*	1.81	1.42	0.39	21.4	〃	〃
22) *Aeodes lanceolata*	2.30	1.52	0.78	33.9	Yokosuka	May 8
23) *Cryptonemia sparsa*	2.55	2.05	0.50	19.6	Yokosuka	May 9
24) *Nemalion pulvinatum*	1.74	1.53	0.21	12.1	Kominato	May 16
25) *Grasilaria Textorii*	3.18	—	—	—	〃	〃
26) *Laurencia concinna*	1.82	—	—	—	〃	〃

higher value compared with the old one. In *Undaria pinnatifida* the sporophyll shows higher ratio than the vegetative frond.

The seasonal variation of the total-, protein-, and non-protein-N contents have been determined with *Porphyra tenera*, *Higikia fusiformis* and *Prasiola japonica* respectively. The results are shown in Tables 1–3 and Figs. 1–3. In general, the contents of these three forms of nitrogen exhibit a marked aseasonal variation. In these three species, the content of total N changes with season in a similar way. But the course of the seasonal variation of protein N and non-protein N in *Prasiola* differs considerably from those of *Porphyra* and *Higikia*.

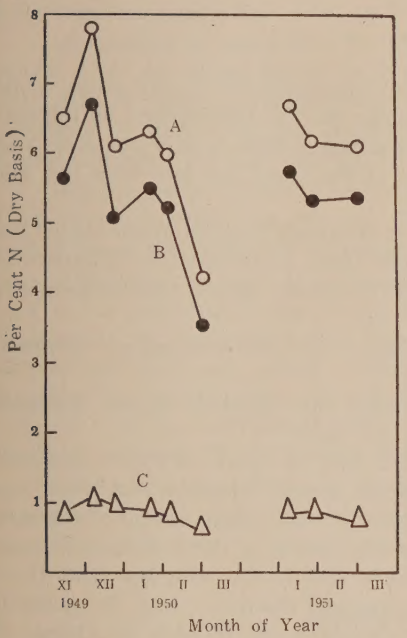

Fig. 1. Seasonal variation in total N, protein N, and non-protein N contents of *Porphyra tenera*. A, total N; B, protein N; C, non-protein N.

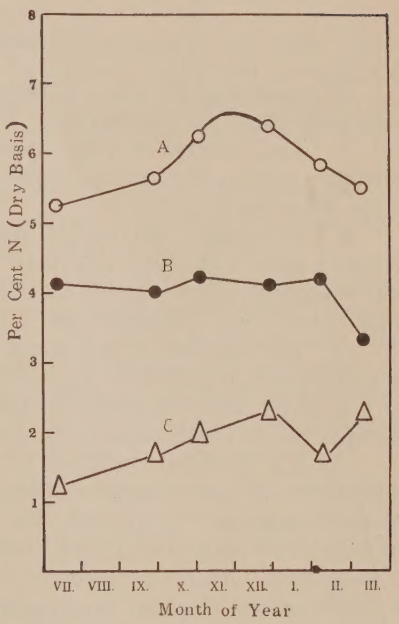

Fig. 2. Seasonal variation in total N, protein N, and non-protein N contents of *Prasiola japonica*. A, total N; B, protein N; C, non-protein N.

Thus, in *Prasiola* the seasonal variation of total N depends chiefly on that of non-protein N, while in *Porphyra* and *Higikia* the change of total N is associated with that of protein N. This phenomenon seems of interest from viewpoint of the nitrogen metabolism of *Prasiola japonica*.

As can be seen in Fig. 1 the contents of total-, protein-, and non-protein-N of *Porphyra tenera* are somewhat different in the same season of the year. A possible explanation may be that these differences may be due to the difference of the content of nutrients in the water.

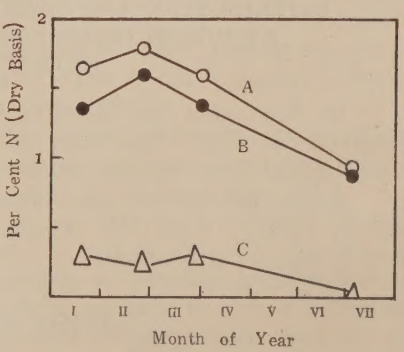

Fig. 3. Seasonal variation in total N, protein N, and non-protein N contents of *Higikia fusiformis*. A, total N; B, protein N; C, non-protein N.

Summary

The contents of total-, protein-, and non-protein-N of marine and some fresh water algae were determined. The results may be summarized as follows:

1. The contents of total-, protein-, and non-protein-N vary not only with individual algae, season of the growth, part of the frond but also with the age in the same species.

2. In general, the total N content is relatively high in green algae, low in brown algae with some exceptions. In red algae, a considerable difference in total N content exists between Bangiales and Florideae, namely, that of Bangiales is high and that of Florideae low.

3. The content of non-protein N varies with different algae. *Prasiola japonica* shows considerably high content.

4. The ratio non-protein N/total N varies with different algae. *Prasiola japonica* shows considerably high value in this ratio.

5. The seasonal variation of the contents of total-, protein-, and non-protein N have been determined with *Porphyra tenera*, *Higikia fusiformis* and *Prasiola japonica*. In general, the contents of these three forms of nitrogen exhibit marked seasonal variation. However the course of the seasonal variation of these three nitrogen fractions in *Prasiola* is considerably different from those of *Porphyra* and *Higikia*. In *Prasiola* the seasonal change of total N proceeds parallel with that of non-protein N but in *Porphyra* and *Higikia* the change of total N is accounted for as due to the variation of protein N.

CHAPTER II

DISTRIBUTION AND SEASONAL VARIATION OF VARIOUS NITROGEN COMPOUNDS IN THE PROTEIN-FREE EXTRACT OF ALGAE

We have thus far but little information about the non-protein nitrogen compounds of algae. The basic N contents in the extract of *Porphyra* and *Enteromorpha* have been determined by FUJIKAWA[1].

In the present investigation the contents of volatile-, amino-, amide-, and basic-N together with their seasonal variation in the protein-free extract of several algae were determined.

Method of Determination

The protein-free extract was prepared in the same manner as described in Chapter I. After the removal of lead, the extract was neutralized to pH 5–6 with dil. sodium hydroxide and made up to 100 ml. The estimation was made with an aliquot of this extract by the following procedures.

Estimation of volatile basic N: 10 ml. of the solution were made alkaline to phenolphthalein with 20 per cent potassium carbonate and the volatile bases were distilled into 0.02 N sulfuric acid at 30°C for 6 hours and titrated with

0.02 N sodium hydroxide.

Estimation of amide N: To 10 ml. of the solution 1 ml. of conc. hydrochloric acid was added, boiled for 2 hours under reflux and after neutralization, again volatile basic N was estimated as described above. Amide N content was calculated by subtracting the original volatile basic N from the volatile basic N determined after the acid hydrolysis.

Estimation of amino N and non-amino N: Amino N was estimated with 2 ml. of the solution by the van Slyke's method. The value of non-amino N was given as the difference of amino N and total N of the extract.

Estimation of basic N and non-basic N: 8 ml. of the solution were measured into a centrifugal tube, 0.5 ml. of 50 per cent sulfuric acid and 1.5 ml. of 25 per cent phosphotungstic acid dissolved in 5 per cent sulfuric acid were added successively and well stirred. After standing for 24 hours the precipitate was separated by centrifugation. Non-basic N was estimated by the Kjeldahl method with 5 ml. of the supernatant fluid. Basic N content was calculated by subtracting both non-basic N and volatile basic N from total N of the extract.

The results are shown in Table 4 and Eigs. 4–5, respectively. The values expressed in this table indicate percentage of each N form of dry frond. The ratio of each N form to non-protein N was calculated from the above results.

Results and Discussion

As shown in the table, the distribution of various nitrogen compounds in the protein-free extract of algae varies not only with individual species, season of the growth but also with the part of the frond in the same species.

Both volatile basic N and amide N distribute widely among algae but their amounts are very small. The content of amide N of *Prasiola japonica* observed in September and November is somewhat higher than those of other species. The amide N present in the extract of algae may be amide compounds such as asparagine or glutamine as in higher plants. But in certain species of brown algae a part of amide N may originate from eisenin[6].

The amino N content in the protein-free extract is generally high throughout all species analyzed. Especially in *Laminaria* sp., the content of amino N amounts to over 90 per cent of the non-protein N and in *Porphyra tenera* it is also very high. Thus, the amino N may be regarded as a principal form of nitrogen in the protein-free extract of algae.

In general, the basic N content in the extract is very low excepting those of several species of green algae. In most species, the basic N content is smaller than that of the non-basic N. In several species of green algae, however, the content of basic N is higher than that of the non-basic N, i.e. in *Prasiola japonica*, *Cladophora* sp., both of which are fresh water algae, and *Ulva pertusa*. Especially in *Prasiola japonica*, the content of basic N atteins its maximum in December, being 1.28 per cent of the dry weight, which corresponds to about 60 per cent of the non-protein N. Accordingly for *Prasiola* the basic nitrogen compounds are the principal ones existing in the protein-free extract. This fact seems to be a characteristic feature in the nitrogen metabolism of this alga as

compared with other species belonging to Phaeophyceae and Rhodophyceae.

As mentioned above, the content of each nitrogen form varies more or less considerably with the part of the frond within the same species. Namely, in *Undaria pinnatifida*, the content of volatile N, amino N and basic N in the sporophyll are higher than those in leaf or stem, and the amide N content in the leaf is higher than that in the sporophyll or stem.

The ratio of each N form to non-protein N is variable for different species. However, in general, each ratio of amino N, non-basic N and non-amino N to non-protein N is relatively high and that of basic N, volatile N and amide N to

Table 4. Distribution and Seasonal Variation of Various N Forms in the Protein-Free Extract of Algae. Figures indicate the per cent N of each fraction on dry basis and the ratio of each fraction N to non-protein N. *, phosphotungstic precipitate N – volatile N; **, non-protein N – phosphotungstic precipitate N; ***, fresh water algae.

Chlorophyceae:

Form of Nitrogen	*Ulva pertusa*		*Enteromorpha intestinalis*		*Enteromorpha compressa*	
Non-Protein N	1.268	100	0.143	100	0.792	100
Volatile N	0.097	7.6	0.009	6.8	0.047	5.9
Amide N	0.000	0.0	0.008	5.2	0.093	11.8
Amino N	0.429	33.8	0.042	29.5	0.538	67.9
Non-Amino N	0.839	66.2	0.101	70.5	0.254	32.1
Basic N*	0.811	64.0	0.048	33.5	0.014	1.7
Non-Basic N**	0.361	28.4	0.084	58.7	0.731	92.4
Date of Collection	Nov. 25		May 16		Feb. 8	

Form of Nitrogen	*Cladophora Wrightiana*		*Cladophora* sp.***		*Cladophora* sp.***	
Non-Protein N	0.606	100	0.722	100	0.423	100
Volatile N	0.065	10.7	0.046	6.4	0.105	24.8
Amide N	0.087	13.2	0.028	3.8	0.006	1.4
Amino N	0.389	64.2	0.237	32.8	0.109	25.8
Non-Amino N	0.217	35.8	0.485	67.2	0.314	74.2
Basic N*	0.044	7.2	0.322	44.5	0.165	39.0
Non-Basic N**	0.497	82.0	0.354	49.1	0.153	36.2
Date of Collection	Nov. 25		Nov. 1		May 8	

Form of Nitrogen	*Prasiola japonica***		*Prasiola japonica***		*Prasiola japonica***	
Non-Protein N	1.165	100	1.596	100	1.975	100
Volatile N	0.065	5.5	0.025	1.5	0.082	4.2
Amide N	0.097	8.3	0.214	13.4	0.205	10.4
Amino N	0.453	38.9	0.413	25.9	0.538	27.2
Non-Amino N	0.712	61.1	1.183	74.1	1.437	72.8
Basic N*	0.404	34.7	0.791	49.6	1.143	57.8
Non-Basic N**	0.696	59.7	0.781	49.0	0.750	37.9
Date of Collection	Jul. 8		Sept. 23		Nov. 3	

Phaeophyceae:

Form of Nitrogen	*Prasiola japonica***		*Prasiola japonica***		*Laminaria* sp.	
Non-Protein N	2.204	100	1.643	100	0.685	100
Volatile N	0.067	3.0	0.073	4.5	0.037	5.4
Amide N	0.015	0.7	0.023	1.4	—	—
Amino N	0.469	21.3	0.812	49.3	0.647	94.5
Non-Amino N	1.735	78.7	0.831	50.7	0.038	5.5
Basic N*	1.286	58.3	0.855	52.0	0.222	3.2
Non-Basic N**	0.851	38.6	0.714	43.4	0.626	91.5
Date of Collection	Dec. 29		Feb. 9		—	

Form of Nitrogen	*Eisenia bicyclis*		*Ecklonia cava*		*Undaria pinnatifida* (leaf)	
Non-Protein N	0.474	100	0.205	100	0.463	100
Volatile N	0.052	11.1	0.011	5.5	0.059	12.7
Amide N	0.025	5.3	0.020	9.8	0.056	12.0
Amino N	0.106	22.4	0.084	40.7	0.202	43.6
Non-Amino N	0.368	77.6	0.121	59.3	0.261	56.4
Basic N*	0.051	10.7	0.045	22.1	0.097	20.9
Non-Basic N**	0.370	78.0	0.149	72.7	0.307	66.4
Date of Collection	Apr. 25		May 9		Apr. 26	

Form of Nitrogen	*Undaria pinnatifida* (sporophyll)		*Undaria pinnatifida* (stem)		*Sargassum Ringgoldianum*	
Non-Protein N	0.660	100	0.485	100	0.661	100
Volatile N	0.099	15.0	0.081	17.7	0.071	10.7
Amide N	0.037	5.6	0.027	5.9	0.078	11.7
Amino N	0.252	38.2	0.177	38.6	0.164	24.8
Non-Amino N	0.408	61.8	0.281	61.4	0.497	75.2
Basic N*	0.123	18.6	0.013	2.8	0.092	13.8
Non-Basic N**	0.438	66.4	0.364	79.4	0.498	75.4
Date of Collection	Apr. 26		Apr. 26		May 9	

Rhodophyceae:

Form of Nitrogen	*Sargassum Thunbergii*		*Ishige Okamurai*		*Chondrus ocellatus*	
Non-Protein N	0.101	100	0.283	100	0.315	100
Volatile N	0.011	11.1	0.023	7.9	0.013	4.2
Amide N	—	—	0.003	1.4	0.006	1.9
Amino N	0.016	15.3	0.090	31.9	0.057	18.0
Non-Amino N	0.086	84.7	0.193	68.1	0.258	82.0
Basic N*	0.026	25.6	0.081	28.6	0.118	37.4
Non-Basic N**	0.065	64.3	0.180	63.6	0.184	58.4
Date of Collection	May 16		May 16		May 9	

Form of Nitrogen	*Aeodes lanceolata*		*Porphyra tenera*		*Porphyra tenera*	
Non-Protein N	0.746	100	0.821	100	1.075	100
Volatile N	0.019	2.6	0.066	8.0	0.078	7.3
Amide N	0.000	0.0	—	—	—	—
Amino N	0.188	25.2	0.533	64.9	0.899	83.5
Non-Amino N	0.558	74.7	0.288	35.1	0.176	16.5
Basic N*	0.271	36.4	0.274	33.4	0.095	8.9
Non-Basic N**	0.455	61.0	0.481	58.5	0.902	83.9
Date of Collection	May 8		Nov. 15		Dec. 8	

Form of Nitrogen	*Porphyra tenera*		*Porphyra tenera*		*Porphyra tenera*	
Non-Protein N	0.975	100	0.865	100	0.814	100
Volatile N	0.027	2.8	0.030	3.5	0.047	5.8
Amide N	—	—	—	—	—	—
Amino N	0.751	77.0	0.686	79.4	0.661	81.2
Non-Amino N	0.224	23.0	0.176	20.6	0.153	18.8
Basic N*	0.079	8.1	0.096	11.2	0.044	5.4
Non-Basic N**	0.869	89.0	0.740	85.5	0.725	89.0
Date of Collection	Dec. 28		Jan. 18		Feb. 5	

Form of Nitrogen	*Porphyra tenera*	
Non-Protein N	0.655	100
Volatile N	0.068	10.3
Amide N	—	—
Amino N	0.429	65.5
Non-Amino N	0.226	34.5
Basic N*	0.034	5.2
Non-Basic N**	0.555	84.7
Date of Collection	Mar. 2	

non-protein N is considerably low except some species of green algae. In green algae thus far examined, i.e., *Prasiola japonica*, *Cladophora* sp., and *Ulva pertusa*, the content of basic N in the protein-free nitrogen extract is large compared with that of red and brown algae.

In *Porphyra tenera* and *Prasiola japonica*, on which the seasonal variation of various nitrogen forms has been examined, noteworthy results have been obtained. In *Porphyra tenera*, the values of non-basic N and amino N are far greater than those of other nitrogen forms, indicating that the main nitrogen compounds in the protein-free extract may probably be monoamino acids. Accordingly the principal nitrogen compound which has been found to vary seasonally and may be regarded to be physiologically important, must be monoamino acids. In the case of *Prasiola japonica*, however, the situation is different from that of *Porphyra tenera*. In this alga, the seasonal course of the variation of the non-amino N and the basic N contents proceeds nearly parallel with that of the non-protein N. No correlation between the content of non-protein N and that

of amino N could be observed, though in its order the content of the latter is next to that of non-amino N or basic N. Accordingly, it may be considered probable that the principal nitrogen compounds in the protein-free extract of this alga might be diamino acids and these might be mainly responsible for the seasonal variation of non-protein N. These facts may suggest some difference in the nitrogen metabolism existing between *Porphyra* and *Prasiola*. So far as the present investigation is concerned, in most species of brown algae the prin-

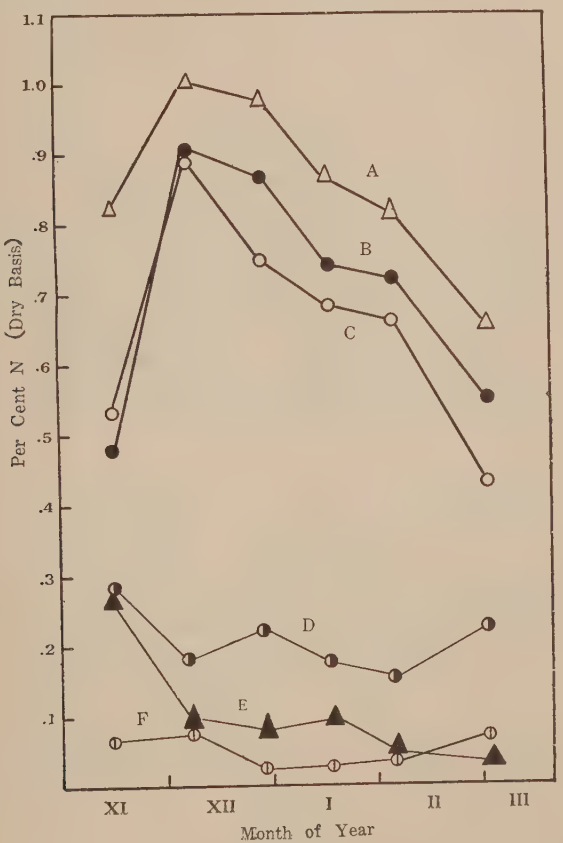

Fig. 4. Seasonal variation of various N forms in the protein-free extract of *Porphyra tenera*. A, non-protein N; B, non-basic N; C, amino N; D, non-amino N; E, basic N; F, volatile N.

cipal nitrogen compound in the non-protein nitrogen fraction may also be monoamino acids as in the case of *Porphyra*, since their amino N contents are relatively high and the basic N contents low. In the case of green algae, however, no such general relation exists, the content of monoamino or diamino acids varying more or less considerably with different species. In Florideae of red algae, the data

obtained are still so insufficient that nothing definitive can be stated. In *Prasiola japonica* the diamino acid appears to play an important part in the nitrogen metabolism.

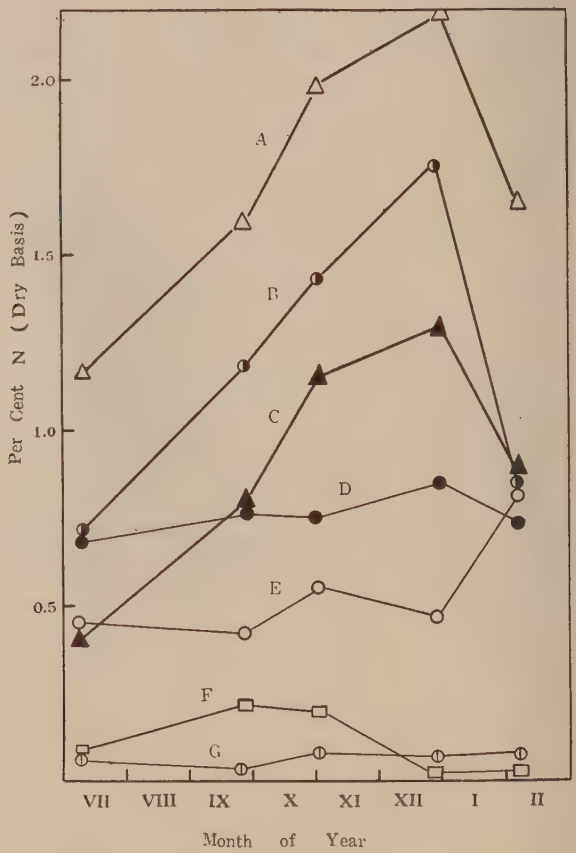

Fig. 5. Seasonal variation of various N forms in the protein-free extract of *Prasiola japonica*. A, non-protein N; B, non-amino N; C, basic N; D, non-basic N; E, amino N; F, amide N; G, volatile N.

Summary

The distribution and seasonal variation of various nitrogen forms in the protein-free extract of marine and a few fresh water algae were estimated. The results can be summarized as follows:

1. The content of various nitrogen forms in the protein-free extract of algae varies with the species, season of the growth as well as with the part of the frond in the same species.

2. The content of volatile N and amide N is small.

3. Amino N is the principal form of nitrogen in the protein-free extract of algae.

4. In general, brown and red algae contain but very small amount of basic N, while in green algae the content of basic N is varying with different species. In *Prasiola japonica*, particularly high content of basic N was observed.

5. The seasonal variation in the content of various nitrogen forms has been determined with *Porphyra tenera* and *Prasiola japonica*. In *Porphyra* the principal nitrogen compound in the protein-free extract was found to be monoamino acids and in *Prasiola* diamino acids. Therefore the non-protein nitrogen compounds which display marked seasonal variation must be monoamino acids in *Porphyra* and diamino acids in *Prasiola*.

CHAPTER III

AMINO ACIDS OF ALGAE

As mentioned in the preceding chapter, the principal nitrogenous constituents of the non-protein fraction of algae are free amino acids. Consequently, it may be expected that a detailed study on the nature of these amino acids would certainly make a valuable contribution to the knowledge of the biochemistry of algae. However we have had as yet very little information in this field.

The purpose of the present study is to elucidate the nature and quantity of amino acids existing in the non-protein fraction and those appearing in hydrolysate of several algae.

(1). Free Amino Acids

With regard to the investigations hitherto made on free amino acids of algae, Ikeda[7] has found glutamic acid in *Laminaria*, Tadokoro and Takasugi[8] have isolated lysine and arginine from *Iridea laminarioides*, and Takagi[9] has shown the existence of histidine and lysine in *Porphyra pseudolinearis*. Recently, on the other hand, the development of the paper partition chromatography[10] has made brilliant successes in providing many significant informations about free amino acids occurring in organisms.[11),12),13]

In the present investigation amino acids in the non-protein fraction of green, brown, and red algae including some fresh water species were studied by paper chromatography.

Method

Samples varying in weight from 0.5 to 2 g were thoroughly extracted with hot water (70°C). To the combined extracts lead acetate was added and filtered. The excess of lead in the filtrate was removed with hydrogen sulfide and the filtrate was concentrated to a small bulk *in vacuo*. In certain samples, the extraction of amino acids was made with 85 per cent alcohol.

Amino acids were identified after the two dimensional method in which the ascending process was adopted. The size of filter paper employed (Toyo filter paper No. 2) was 20 cm. square.

A test solution containing about 40γ of non-protein N was placed from a

capillary pipette at a point 3 cm. from the paper ends. Phenol-0.1 per cent ammonia mixture (5:1) was used as the first developing solution. *n*-Butyl alcohol, a mixture of *n*-butyl alcohol and glacial acetic acid (4:1), a mixture of *n*-butyl alcohol and 95 per cent ethyl alcohol (4:1) or collidine was used respectively as second solvent. *n*-Butyl alcohol and collidine (b. p. 167-177°C) saturated with water were used, the latter solvent being adjusted to pH 6.0 with 50 per cent

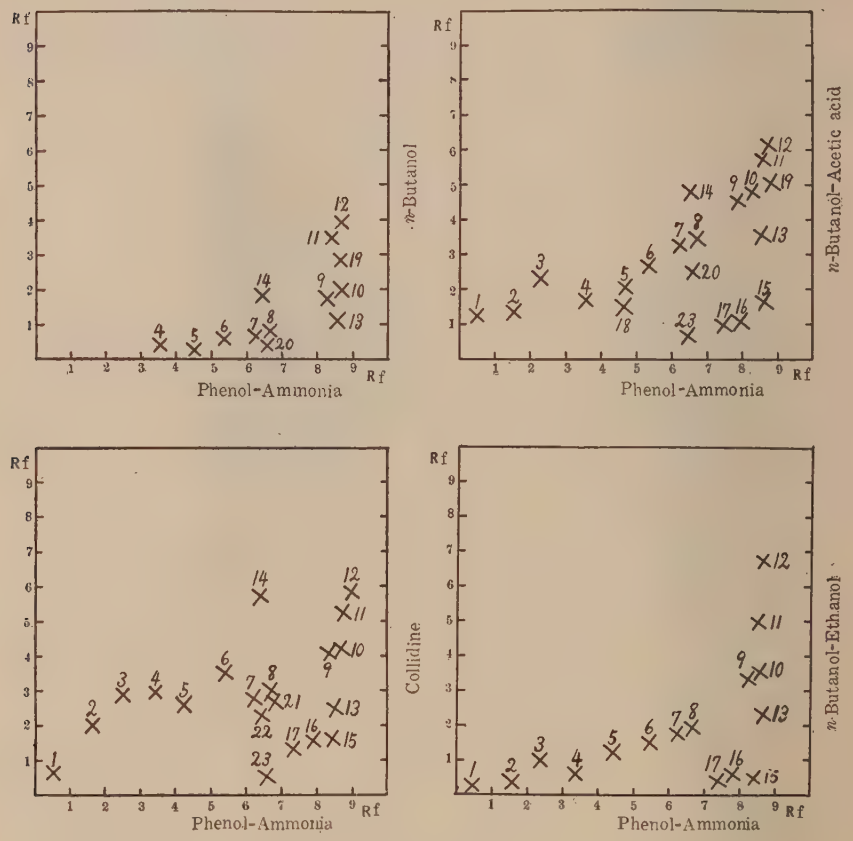

Fig. 6. Standard chromatograms of amino acids obtained with various developing agents.

Numbers in the figures indicate following amino acids:

1	Cystine	9	Valine	17	Lysine
2	Aspartic acid	10	Methionine	18	Asparagine
3	Glutamic acid	11	Leucine	19	Tryptophan
4	Serine	12	Phenylalanine	20	Glutamine
5	Glycine	13	Proline	21	β-Alanine
6	Threonine	14	Tyrosine	22	Citrulline
7	Alanine	15	Arginine	23	Ornithine
8	Hydroxyproline	16	Histidine		

acetic acid[13]. Ninhydrin was used for the development of the color. By the size of the spot and the intensity of its color approximate quantity of amino acids can be roughly estimated. Diamino acids were tested after precipitation with phosphotungstic acid. Arginine and histidine were detected respectively by Sakaguchi's reaction and diazo reaction. Thus the standard chromatograms were prepared with pure amino acids as well as with casein hydrolysate (Fig. 6) and they were compared with the chromatograms of amino acid mixture from algae. The results are shown in Table 5.

Results and Discussion

As seen in the table, of monoamino acids glutamic acid, aspartic acid, alanine, and cystine are the most common in their distribution thus far observed. Of diamino acids, arginine occurs most widely, and histidine also shows a distribution of considerable wideness. The occurrence of glycine, leucine, serine, threonine, phenylalanine, tyrosine, valine, proline, hydroxyproline, and tryptophan is more or less fluctuating. Methionine could not be detected, while the presence of ornithine is evident in some of green algae. Other amino acids having been considered as β-alanine or citrulline are also present in a green alga, *Enteromorpha Linza*. As regards amide compounds asparagine is recognized in green algae. Though glutamine could not be verified in its existence, it cannot be simply stated that it is absent in algae, as this amide is so unstable that it might have been decomposed during the course of the treatment. The amino acids belonging to the non-protein fraction vary slightly, if at all, with the season of the growth as well as with the part of the frond in the same species. Thus, in *Prasiola japonica*, the presence of aspartic acid and valine can be noticed only with the sample collected in December. Their distribution differs somewhat with the leaf, sporophyll and with the stem in the case of *Undaria pinnatifida*.

As already mentioned in the preceding chapter, the principal nitrogen compound in the non-protein fraction of *Porphyra tenera* is monoamino acids, while that of *Prasiola japonica* is diamino acids. The present study revealed that monoamino acids of *Porphyra* were glycine, alanine, leucine, serine, threonine, aspartic acid, glutamic acid, valine, and cystine, of which glutamic acid and alanine were present in larger quantity. These amino acids, especially glutamic acid and alanine seem to be primarily responsible for the seasonal variation of non-protein nitrogen. *Prasiola japonica* contains a large amount of arginine and is rich in both glutamic acid and alanine as well. Accordingly, the seasonal variation of the non-protein nitrogen may be mainly attributed to that of arginine.

As regards the genesis of amino acids in algae, little is known as to the pathway through which amino acids are formed[14]. In this respect the results of the present study that glutamic acid, aspartic acid and alanine are present not only in appreciable quantity but also very widely throughout various algae, seems of special interest. The abundance of free arginine in *Prasiola* might suggest the biochemical importance of this amino acid in this alga.

Table 5. Qualitative Test for Free Amino Acids of Algae.
+, existent; ‡, a large quantity; ‡‡, a very large quantity; (+), existent (probably); −, absent; *, fresh water algae.

Name of Algae	Glycine	Alanine	Leucine & Isoleucine	Serine	Threonine	Phenylalanine	Tyrosine	Aspartic acid	Glutamic acid	Valine	Cystine	Methionine	Proline	Hydroxyproline	Tryptophan	Arginine	Histidine	Lysine	Asparagine	Glutamine	β-Alanine	Citrulline	Ornithine	Date of Collection
Chlorophyceae:																								
*Prasiola japonica**	+	‡	−	−	−	−	−	−	‡	−	−	−	−	−	−	+	−	−	+	−	−	−	+	Aug. 21
*Prasiola japonica**	+	‡	(+)	+	−	−	−	−	‡	−	−	−	−	−	−	‡	+	−	+	−	−	−	+	Sep. 23
*Prasiola japonica**	+	‡	+	+	−	−	−	+	‡	+	−	−	(+)	−	−	‡	+	−	+	−	−	−	+	Dec. 29
Enteromorpha Linza	+	‡	+	−	−	−	−	+	‡	+	+	−	−	−	(+)	+	+	−	+	−	+	+	−		
E. intestinalis	−	‡	−	−	+	−	−	+	+	−	+	−	−	−	−	+	+	−	−	−	−	−	−		
E. compressa	−	+	−	+	−	−	−	+	‡	−	+	−	−	−	−	+	−	−	+	−	−	−	(+)		
Ulva pertusa	+	+	−	+	−	−	−	−	+	−	+	−	−	−	−	+	−	−	−	−	−	−	(+)		
Cladophora Wrightiana	−	‡	+	(+)	(+)	+	−	+	+	+	+	−	(+)	−	−	+	−	−	(+)	−	−	−	−		
Cladophora sp.*	+	‡	+	+	+	+	+	+	‡	−	+	−	−	+	−	+	+	+	+	−	−	−	−		
Phaeophyceae:																								
Laminaria sp.	−	‡	−	−	−	−	−	‡	‡‡	−	+	−	+	−	−	+	−	−	−	−	−	−	−		
Ecklonia cava	−	‡	−	−	−	−	−	+	+	−	+	−	(+)	−	−	+	+	−	−	−	−	−	−		
Eisenia bicyclis	−	+	−	−	−	−	−	+	+	−	+	−	(+)	−	−	+	+	−	−	−	−	−	−		
Higikia fusiformis	−	+	−	−	−	−	−	+	+	−	+	−	−	−	−	+	−	−	−	−	−	−	−		
Ishige Okamurai	−	+	+	+	−	−	−	−	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−		
Undaria pinnatifida (leaf)	−	‡	−	−	(+)	−	+	+	+	−	+	−	−	−	−	+	−	−	−	−	−	−	+		
〃 〃 (sporophyll)	(+)	‡	−	(+)	−	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−		
〃 〃 (stem)	−	‡	+	−	(+)	(+)	−	+	+	+	+	−	−	(+)	−	+	+	−	−	−	−	−	−		
Sargassum Thunbergii	+	+	−	−	−	−	−	+	+	−	+	−	−	−	−	+	−	−	−	−	−	−	−		
S. Ringgoldianum	−	+	−	−	−	−	−	+	+	−	+	−	−	−	−	+	+	−	−	−	−	−	−		
Rhodophyceae:																								
Porphyra tenera	+	‡	+	+	+	−	−	+	‡	−	+	−	−	−	−	+	+	+	−	−	−	−	−	Nov. 22
Porphyra tenera	+	‡	+	+	−	−	−	+	‡	+	+	−	−	−	−	+	+	+	−	−	−	−	−	Jan. 15
Aeodes lanceolata	−	+	−	−	−	−	−	+	+	−	−	−	−	−	−	‡	+	+	−	−	−	−	−		
Chondrus ocellatus	−	+	−	+	−	−	−	−	+	−	+	−	−	−	−	+	−	+	−	−	−	−	−		
Gloiopertis furcata	−	+	−	(+)	−	−	−	(+)	+	−	−	−	−	−	−	+	−	(+)	−	−	−	−	−		
Gigartina intermedia	−	+	−	+	−	−	−	−	+	−	+	−	−	−	−	+	+	−	−	−	−	−	−		
Grateloupia filicina	−	+	−	−	−	−	−	+	+	−	+	−	−	−	−	+	−	−	−	−	−	−	−		
Corallina officinalis	+	+	−	+	−	−	−	+	+	−	+	−	(+)	−	−	+	−	−	−	−	−	−	−		

Summary

The presence and distribution of free amino acids in algae were investigated by means of paper chromatography. The results are summarized as follows:

1. Of monoamino acids, glutamic acid, aspartic acid, alanine, and cystine are distributed most widely. The occurrence of other amino acids varies with species.
2. Of diamino acids, arginine is most commonly found.
3. The existence of asparagine is limited to some green algae.
4. The major amino acids of *Porphyra tenera* are glutamic acid and alanine, those of *Prasiola japonica* are arginine, glutamic acid, and alanine and those of *Laminaria* sp. glutamic acid, aspartic acid and alanine.
5. A considerably large quantity of arginine is present in *Prasiola japonica*.

(2). Arginine of Algae

The present investigation was made with a purpose of elucidating the physiological significance of arginine in algae, especially in *Prasiola japonica*.

(A). Isolation of Arginine from the Extract of *Prasiola japonica* and *Ulva pertusa*.

(i). *Prasiola japonica*: 30 g of the sample were extracted at 70°C three times each with 1000 ml. of water for 30 minutes. The extracts and washings were combined, and filtered after the addition of lead acetate and lead subacetate. The excess of lead in the filtrate was removed with sulfuric acid. The filtrate was neutralized to pH 5 by adding a dilute solution of sodium hydroxide and concentrated to a small bulk *in vacuo*. The bases in the extract were then precipitated with phosphotungstic acid as usual. After standing for 24 hours, the precipitate was filtered off on Büchner funnel and washed with 2 per cent phosphotungstic acid dissolved in 5 per cent sulfuric acid. The precipitate was decomposed with baryta as usual and the bases in arginine fraction were precipitated after Block's method[15] with silver nitrate and baryta. The precipitate was filtered off, suspended in water, decomposed with hydrogen sulfide, and filtered. The filtrate was concentrated *in vacuo* and flavianic acid was added. A considerably large amount of arginine flavianate appeared immediately. The flavianate was recrystallized from boiling water. The product was washed with cold water, alcohol, and ether successively and dried. The yield of arginine flavianate was 0.685 g, corresponding to 0.81 per cent of free arginine in the sample. After one

Table 6. Nitrogen Content of Arginine Flavianate Isolated from *Prasiola japonica*. Nitrogen was determined by Kjeldahl method after reducing the flavianate with zinc dust and hydrochloric acid.

Sample	N/50 H_2SO_4	N	
		Found	Calculated
18.5 mg.	11.23 ml.	17.03%	17.21%
15.7	9.50	16.94	

more recrystallization from hot water the flavianate melted at 258°C with decomposition. The nitrogen content of the flavianate is shown in Table 6.

A part of flavianate was dissolved in boiling water, treated with baryta, and filtered. The filtrate was neutralized immediately to pH 5 with sulfuric acid, decolorized with norit, and filtered. The filtrate was concentrated on a boiling water bath. For the identification of arginine in this solution Rf values on the paper chromatogram were compared with those of an authentic specimen of this amino acid each with phenol, phenol-ammonia and collidine as developing agent. The results shown in Table 7 indicate a complete coincidence of Rf values between amino acid in the test solution and pure arginine.

Table 7. Rf Values on Paper Chromatogram of Arginine Isolated from *Prasiola japonica* and of Pure Arginine against Several Developing Agents (18°C). Developing agents used were the same as described above (p. 122).

	Phenol	Phenol+Ammonia	Collidine
Sample	0.42	0.80	0.20
Pure Arginine	0.42	0.80	0.20

(ii). *Ulva pertusa*: 2 kg of the sample (moisture 20% and sand 15%) were extracted with hot water several times. The combined extracts were treated as in *Prasiola*. Arginine was converted to flavianate with a yield of 0.15 g. The nitrogen content of the flavianate is shown in Table 8. The flavianate was dissolved in boiling water, treated with baryta, and filtered. The filtrate was neutralized to pH 5 with sulfuric acid, decolorized with norit, and filtered. The final filtrate thus obtained showed a positive Sakaguchi's reaction strongly.

Table 8. Nitrogen Content of Arginine Flavianate Isolated from *Ulva pertusa*. Nitrogen was estimated by Kjeldahl method after reducing the flavianate with zinc dust and hydrochloric acid.

Sample	N/10 H_2SO_4	N	
		Found	Calculated
0.1079 g	12.99 ml.	16.89%	17.21%

(B). The Contents of Free and Combined Arginine in Algae

Method for the Determination of Arginine

Numerous investigations have been reported on the method of the determination of arginine[15)-23)]. In the present study the Weber's colorimetric method[22)] which is based on the Sakaguchi's reaction[23)] was adopted. This method has been regarded as suitable for the determination of arginine in plant materials[24),25)].

Determination of free arginine: Samples varying in weight from 0.1 to 2 g were extracted exhaustively with water at 80°C. The extracts and washings were combined and purified with lead acetate. After removal of the excess of

lead with sulfuric acid the filtrate was made up to 100 ml. An aliquot of this solution, corresponding to 0.01–0.2 mg of arginine, was measured into a test tube and made up to 5 ml. with water. The test tube was cooled in ice water and 1 ml. of 25 per cent sodium hydroxide and 1 ml. of 0.02 per cent alcoholic α-naphthol solution were added successively and well mixed. After cooling for 3 minutes, 0.1–0.5 ml. of sodium hypobromite prepared by dissolving 2 g of bromine in 100 ml. of 5 per cent sodium hydroxide was added with stirring and immediately followed by the addition of 1 ml. of 40 per cent urea solution. After cooling for 5 minutes the color developed was compared with that of the standard using a Dubosq type colorimeter. Volume of sodium hypobromite solution required had been determined previously.

The standard solution was prepared so as to contain 0.01 mg of arginine or arginine salt in 1 ml. 1–5 ml. of the standard solution was measured into a test tube, made up to 5 ml. with water, cooled in ice water, and was treated in the same manner as described above, but in this case 10 per cent sodium hydroxide was used instead of 25 per cent solution.

Determination of total arginine: 0.1–1 g of the sample were hydrolysed with 20 per cent hydrochloric acid (20–30 ml.) by boiling under reflex for 20 hours, after which a greater part of the hydrochloric acid in hydrolysate was neutralized with sodium carbonate. The hydrolysate was then decolorized with a small amount of norit, filtered, and the residue was washed several times with hot water. The filtrates and washings were combined, concentrated *in vacuo*, and

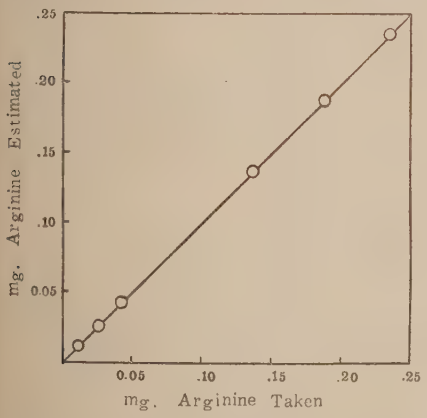

Fig. 7. Relation between color-intensity and arginine concentration.

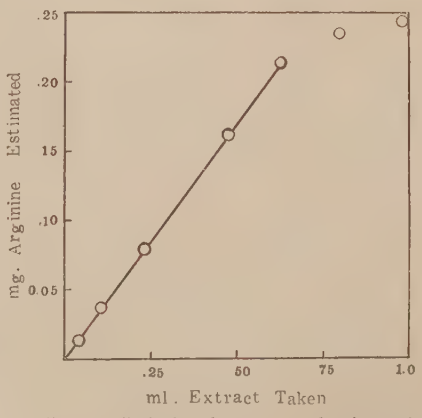

Fig. 8. Relation between color-intensity and arginine concentration. Sample solution used is the extract of *Prasiola japonica* (1.4846 g in 100 ml.).

made up to 200 ml. An aliquot corresponding to 0.01–0.2 mg of arginine was measured into a test tube, made up to 5 ml. with water, and determined the arginine in the same manner as described in free arginine determination. Combined arginine content was obtained by subtracting free arginine from total arginine.

Accuracy of the method: The relation between color intensity and arginine concentration was determined with pure arginine solution and with the extract of *Prasiola*. In both cases the color intensity was proved to be perfectly proportional to the concentration of arginine within a range of 0.01–0.2 mg in 5 ml. of the test solution (Figs. 7 and 8). Arginine added to a sample was recovered with an error below 6 per cent in free arginine determination and 10 per cent in total arginine determination (Tables 9 and 10).

Table 9. Recovery of Arginine Added to a Sample in Free Arginine Determination (Sample: *Prasiola japonica*).

Sample	Arginine added	Arginine found	Arginine recovered	Recovery
0.3625 g	0 mg	9.06 mg	— mg	— %
0.3625	0.91	10.02	0.96	105.5
0.3625	0.91	10.03	0.97	106.5

Table 10. Recovery of Arginine Added to a Sample in Total Arginine Determination (Sample: *Prasiola japonica*).

Sample	Arginine added	Arginine found	Arginine recovered	Recovery
0.1276 g	0 mg	6.672 mg	— mg	— %
0.1276	0.455	7.085	0.413	90.9

In this way the content of free and combined arginine was determined with a number of algae and the results are presented in Table 11. The ratio of total arginine N to total N, the content of arginine in protein are also recorded in the table.

Results and Discussion

Regardless of some exceptions, the content of total arginine is generally high in green and red algae and low in brown algae. However in the case of green algae as well as red algae, it varies appreciably from species to species.

As regards the free arginine content, green algae are much superior to brown and red algae. However, *Cladophora Wrightiana* of green algae shows a low value of the free arginine content, whereas *Aeodes lanceolata* and *Cryptonemia sparsa* of red algae are relatively rich in this amino acid. In *Prasiola japonica*, a large quantity of arginine exists in a free state. The presence in this alga of free arginine can be seen from the fact that no difference in the arginine content could be observed between an air dried sample and one preserved in alcohol immediately after collection (Nos. 13 and 14 of Table 11). In the latter procedure the secondary formation of this amino acid from combined forms can certainly be excluded. The abundance of free arginine in *Prasiola* appears to be of special interest in physiological respect.

The ratio total arginine N/total N is generally high in green and red algae

Table 11. Total-, Free-, and Combined-Arginine Contents of Algae in Per Cent on Dry Basis. *, fresh water algae.

Name of Algae	Total Arginine in Sample	Free Arginine in Sample	Combined Arginine in Sample	Total Arginine N / Total N ×100	Arginine Content in Protein	Date of Collection
Phaeophyceae:						
1) *Laminaria* sp.	0.245	trace	0.245	4.69	3.92	—
2) *Eisenia bicyclis*	0.517	0.0336	0.483	7.33	4.30	Apr. 25
3) *Ecklonia cava*	0.376	0.0107	0.365	7.42	4.11	May 9
4) *Undaria pinnatifida* (leaf)	0.598	0.0345	0.563	10.74	6.77	Apr. 26
5) 〃 〃 (sporophyll)	0.663	0.0489	0.614	9.56	6.26	〃
6) 〃 〃 (stem)	0.334	0.0329	0.301	6.92	4.40	〃
7) *Cystophyllum sisymbrioides*	0.590	0.0105	0.579	10.37	5.90	May 10
8) *Sargassum Ringgoldianum*	0.414	0.0207	0.393	6.06	4.08	May 9
9) *Sargassum hemiphyllum*	0.631	0.0599	0.571	8.50	4.57	May 10
10) *Sargassum enerve*	0.375	—	—	—	—	Aug.
11) *Sargassum Thunbergii*	0.681	0.0094	0.672	10.37	5.36	May 16
12) *Ishige Okamurai*	0.553	0.0073	0.546	9.09	5.20	〃
Chlorophyceae:						
13) *Prasiola japonica**	2.737	1.271	1.466	18.97	6.46	Aug. 21
14) 〃 〃	—	1.236	—	—	—	〃
15) 〃 〃	3.183	1.579	1.604	19.46	6.52	—
16) *Cladophora* sp.*	1.566	0.212	1.354	9.19	4.56	Nov. 11
17) 〃 〃	1.418	0.209	1.209	8.89	4.12	May 8
18) *Cladophora Wrightiana*	—	0.030	—	—	—	Nov. 25
19) *Ulva pertusa*	0.817	0.144	0.673	9.81	4.33	—
20) 〃 〃	—	0.848	—	—	—	Nov. 25
21) *Enteromorpha Linza*	2.832	0.470	2.362	15.93	7.72	Feb. 20
22) *Enteromorpha intestinalis*	0.517	0.242	0.275	12.96	3.86	May 16
23) *Enteromorpha compressa*	1.746	0.401	1.345	12.66	5.92	Feb. 2
Rhodophyceae:						
24) *Porphyra tenera*	2.581	0.0961	2.485	13.42	7.43	Jan. 26
25) *Gelidium elegans*	0.494	0.0209	0.473	9.47	5.18	—
26) *Chondrus ocellatus*	0.957	0.0344	0.923	11.11	6.03	May 9
27) 〃 〃 *f. canaliculata*	0.819	—	—	14.80	—	May 29
28) *Aeodes lanceolata*	1.821	0.987	0.834	25.47	8.78	May 8
29) *Grateloupia elliptica*	0.938	0.020	0.918	14.42	7.45	〃
30) *Cryptonemia sparsa*	1.204	0.315	0.889	15.20	6.93	May 9
31) *Grateloupia filicina*	0.730	0.012	0.718	11.35	6.46	May 16
32) *Grateroupia ligulata*	0.901	—	—	12.42	—	May 29

Table 11. (Continued)

Name of Algae	Total Arginine in Sample	Free Arginine in Sample	Combined Arginine in Sample	Total Arginine N / Total N ×100	Arginine Content in Protein	Date of Collection
33) *Gigartina intermedia*	0.660	0.036	0.624	11.72	7.04	May 29
34) *Gloiopertis cervicornis*	1.023	0.028	0.995	13.43	7.54	May 16
35) *Nemalion pulvinatum*	0.546	0.032	0.514	10.08	5.38	〃
36) *Laurencia concinna*	0.619	—	—	10.94	—	〃
37) *Gracilaria Textorii*	0.965	—	—	9.76	—	〃
38) *Corallina officinalis*	0.267	0.042	0.225	13.46	6.74	〃
39) *Gloiopertis furcata* (young frond)	0.869	0.029	0.840	11.12	6.72	〃
40) 〃 〃 (sporophyll)	0.884	0.033	0.851	11.35	6.63	〃
41) 〃 〃 (old frond)	0.676	0.018	0.658	12.80	7.25	〃

and low in brown algae. The value is relatively high in *Aeodes lanceolata*.

The arginine content of algae varies not only with the part as well as with the age of frond but also with the season of growth in the same species. Thus, in *Undaria pinnatifida*, the sporophyll shows higher values in both free and combined arginine compared with the leaf and stem. In *Gloiopeltis furcata* the young frond shows higher values in both free and combined arginine than old one.

(C). Seasonal Variation in Arginine Content of *Prasiola japonica* and *Porphyra tenera*

Seasonal variations in the content of free and combined arginine were investigated with *Prasiola japonica* and *Porphyra tenera*. The results are shown in Table 12 and Figs. 9 and 10.

Results and Discussion

In *Prasiola japonica*, the content of total arginine calculated on dry basis increases slowly from July to September, after which it increases rapidly and reaches a maximum of 5.3 per cent in December and then continues to decrease gradually till following year.

The free arginine content begins to increase progressively from July, attaining a maximum of 2.6 per cent in December and then decreases gradually till March. In December half of the total arginine exists in a free state.

With regard to the combined arginine content no variation can be observed from July to September but after that it increases gradually and arrives at a maximum of 2.7 per cent in February. But from December to March no appreciable change is found in the combined arginine content. Accordingly the seasonal variation of total arginine seems to be determined primarily by the

variation of free arginine.

Free arginine N which is expressed as per cent of non-protein N and of basic N are shown in Table 13. In its maximum the content of free arginine N corresponds to 39 per cent of non-protein N and 67 per cent of basic N. Consequently, in *Prasiola*, the seasonal variation of non-protein N (see Fig. 2, p. 113)

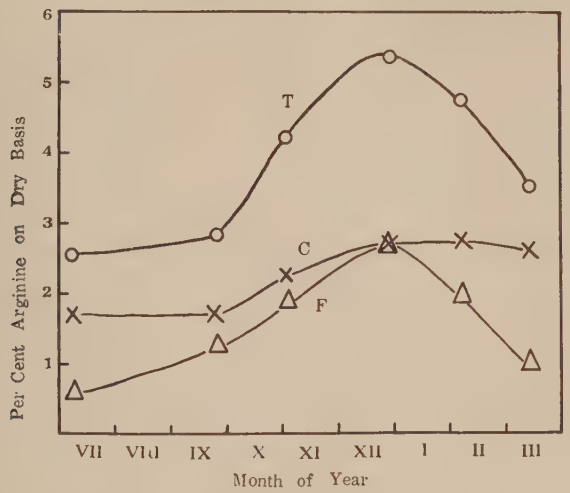

Fig. 9. Seasonal variation in arginine content of *Prasiola japonica*. T, total arginine; C, combined arginine; F, free arginine.

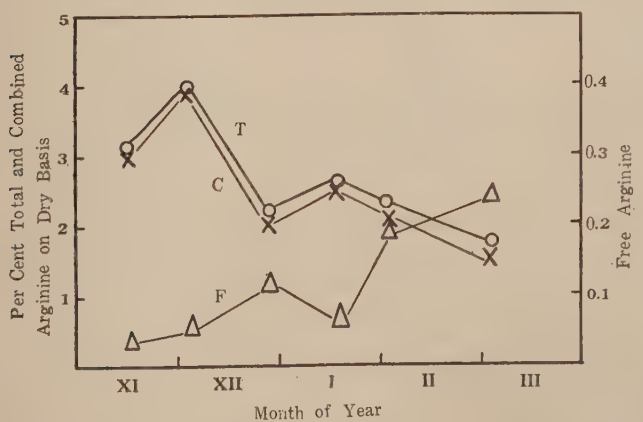

Fig. 10. Seasonal variation in arginine content of *Porphyra tenera*. T, total arginine; C, combined arginine; F, free arginine.

may proceed principally parallel with the seasonal variation of free arginine content. These features might suggest some important role of arginine played in the nitrogen metabolism of this alga.

In *Porphyra tenera*, the mode of the seasonal variation of arginine is quite different from that of *Prasiola*. In this alga, the variation of total arginine is parallel to that of combined arginine throughout all seasons, whereas the content of free arginine remains very low and varies rather inversely to combined arginine. Thus it appears probable that the variation of free arginine might be related to the formation and break down of protein.

Table 12. Seasonal Variation in Arginine Content of *Prasiola japonica* and of *Porphyra tenera* (% on dry basis).

Date of Collection	Total Arginine	Free Arginine	Combined Arginine
Prasiola japonica:			
Jul. 8	2.313	0.566	1.747
Sept. 23	2.849	1.203	1.646
Nov. 3	4.119	1.857	2.262
Dec. 29	5.372	2.686	2.686
Feb. 9	4.755	1.968	2.787
Mar. 11	3.483	0.939	2.544
Porphyra tenera:			
Nov. 15	3.121	0.0311	3.090
Dec. 8	3.963	0.0515	3.911
Dec. 28	2.180	0.114	2.066
Jan. 18	2.635	0.0605	2.574
Feb. 5	2.326	0.193	2.133
Mar. 2	1.826	0.241	1.585

Table 13. Seasonal Variation in Free Arginine N of *Prasiola japonica*.

Date of Collection	Free Arginine N	Free Arginine N / Non-Protein N	Free Arginine N / Basic N
Jul. 8	0.182%	15.6%	45.2%
Sept. 23	0.387	24.3	48.6
Nov. 3	0.598	30.2	52.3
Dec. 29	0.865	39.2	67.2
Feb. 9	0.643	38.6	74.2
Mar. 11	0.302	13.4	—

(D). Discussion on Physiology of Arginine in *Prasiola japonica*

Judging from the above investigations it seems reasonable to consider that arginine plays a significant role in the nitrogen metabolism of *Prasiola japonica*.

According to FUJIYAMA[26] *Prasiola* performs sexual reproduction in winter, when a noticeable accumulation of arginine was found to occur. A coincidence of these two phenomena seems physiologically important. If high arginine content is connected with the phenomenon of reproduction, it is conceivable that *Prasiola* may probably synthesize in winter protamine or histone like substance, which is characterized by the high content of arginine, for the formation of reproductive cells. In the case of higher plants the occurrence of protamine in the pollens of *pine tree* and of *hemp palm*, and the spore of *Lycopodium* have been reported by MALTINO[27] and FULCHIGNONI[27] [28]. If *Prasiola* synthesizes such a substance for the formation of spore, the higher concentration of arginine in winter may be interpreted.

Another explanation may be that in this alga arginine may possess the same physiological function as that of asparagine or glutamine in higher plants. The seasonal variation of free arginine N and amide N of this alga are illustrated in Fig. 11. As seen from the figure, some relations seem to exist between the variation of these two forms of nitrogen. Namely, the variation of free arginine

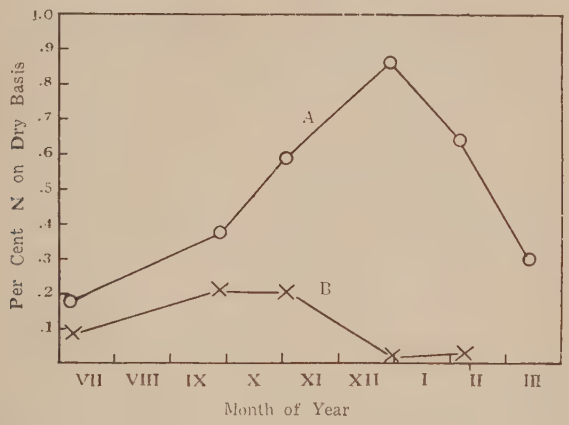

Fig. 11. Seasonal variation of free arginine N and amide N of *Prasiola japonica*. A, free arginine N; B, amide N.

N proceeds rather inversely to that of amide N. In December, free arginine N arrives at a maximum, where amide N is at a minimum. Thus it seems not unlikely that the role played by amide compounds at an early stage of growth may be replaced by arginine at a later stage.

(3). Free Histidine

(A). Isolation of Histidine from the Extract of *Sargassum Ringgoldianum*

2.4 kg of the sample were extracted three times with hot water (80°C). The combined extracts and washings were purified with lead acetate. The excess of lead in the filtrate was precipitated with hydrogen sulfide and filtered. The filtrate was concentrated to a small bulk under reduced pressure and then the basic amino acids were precipitated with phosphotungstic acid as usual. The precipitate was collected on Büchner funnel, washed with 2.5 per cent phosphotungstic acid dissolved in 5 per cent sulfuric acid, and then decomposed with baryta. The excess of baryta was precipitated by passing a stream of carbon dioxide and filtered. The filtrate was concentrated to a small volume *in vacuo*. After removing the purine fraction with silver nitrate, the histidine-arginine fraction was precipitated with silver nitrate and baryta after Kossel-Kutscher's method[29]. The precipitate was filtered off, suspended in water, decomposed with hydrogen sulfide, and filtered. The filtrate was concentrated *in vacuo*, made alkaline with sodium carbonate, and then histidine was precipitated with saturated mercuric chloride solution in 50 per cent alcohol[30]. The precipitate was filtered off, decomposed with hydrogen sulfide, and filtered. The filtrate was

concentrated *in vacuo* and then histidine was converted to hydrochloride. A small amount of histidine hydrochloride was collected, dissolved in water, and converted to picrate. The histidine picrate was recrystallized from water, yielding 0.197 g. The nitrogen content of the picrate, as shown in Table 14, agreed with that of histidine monopicrate.

Table 14. Nitrogen Content of Histidine Picrate Isolated from *Sargassum Ringgoldianum*.
Nitrogen was determined by Kjeldahl method after reducing the picrate with zinc dust and hydrochloric acid.

Sample	N/10 H_2SO_4	N	
		Found	Calculated
0.1259 g	19.42 ml.	21.51%	21.85%

(B). The Content of Free Histidine

Method for the Determination of Histidine

Samples varying in weight from 10 to 30 g were extracted three times (600 ml. in each) with hot water (80°C). To the combined extracts and washings lead acetate and lead subacetate were added and filtered. After removal of the excess of lead with sulfuric acid, the filtrate was concentrated to a small bulk *in vacuo* and histidine was precipitated by adding 10 per cent mercuric sulfate dissolved in 5 per cent sulfuric acid[31]. After standing for 24 hours the precipitate was filtered off, decomposed with hydrogen sulfide, and filtered. The combined filtrates and washings were decolorized with a small amount of norit, concentrated *in vacuo*, and made up to 100 ml. The histidine was determined

Table 15. Free Histidine Content in Per Cent on Dry Basis.
*, fresh water algae.

Chlorophyceae:	
*Prasiola japonica**	0.004
Enteromorpha Linza	0.016
Enteromorpha intestinalis	trace
Cladophora sp.*	0.017
〃 〃	0.016
Phaephyceae:	
Laminaria sp.	0.018
Eisenia bicyclis	0.037
Ecklonia cava	0.013
Sargassum Ringgoldianum	0.012
Sargassum Thunbergii	trace
Sargassum hemiphyllum	0.002
Cystophyllum sisymbricides	0.010
Ishige Okamurai	0.004
Rhodophyceae:	
Aeodes lanceolata	0.001
Chondrus ocellatus	0.004
Gigartina intermedia	0.002
Corallina officinalis	trace

colorimetrically by diazo method[32] using 1 ml. of the solution. The results obtained with several species belonging to green, brown and red algae are shown in Table 15.

Results

As shown in the table, histidine is a very common amino acid but its content is by no means abundant and varies according to the species of algae.

(4). Distribution of Several Forms of Nitrogen in the Hydrolysate of Whole Algae

In the preceding studies, the free amino acids of algae have been investigated with regard to their kind, content and seasonal variation from the viewpoint of biochemistry. In the present studies, in turn, the investigation has been focused on the amino acids composing the protein of algae. To make clear the amino acid component and the nature of protein it seems necessary to investigate the nitrogen distribution in the hydrolysate of algae.

As regards the distribution of various forms of nitrogen, several investigations have been made with the hydrolysate of whole algae or crude protein isolated from algae. Namely the following algae have been analyzed by Hausmann's method or van Slyke's method, viz., *Laminaria* sp.[33], *Undaria pinnatifida*[34], *Enteromorpha* sp.[33], and *Porphyra tenara*[35]. Mazur and Clarke[36] have determined 16 kinds of amino acids, ammonia, and humin N respectively with the hydrolysate of crude proteins of *Laminaria, Sargassum, Chondrus* and *Ulva*. These investigations showed the absence of methionine in the proteins of all these algae and that of tyrosine in *Ulva*, as well as the lack of lysine in *Ulva* and *Laminaria*. Tadokoro and Ugazin[37] have determined the cystine content of algae and shown that brown algae are rich in this amino acid compared with green and red algae. The amino acid composition of phycochromoproteid was determined by Kitazato[38] with phycoerythrine of *Porphyra*, and by Tadokoro[39] with phycoerythrine of *Iridea laminarioides*. Recently Takagi[40] isolated water soluble, salt soluble and alkali soluble proteins from *Ulva pertusa* and determined the nitrogen distribution by van Slyke's method. He also examined several species of *Porphyra* and showed that in all cases arginine N is the principal form of nitrogen in diamino acid fraction[5].

In the present investigation the author has tried to estimate the nitrogen distribution by van Slyke's method in the hydrolysate of whole algae. The difference due to the part of the frond as well as the age in the same species were taken into consideration.

Method

Samples varying in weight from 3 to 10 g were hydrolysed by boiling with 20 per cent hydrochloric acid under reflux for 24 hours. The hydrolysate was filtered and the insoluble residues were washed several times with hot water. The nitrogen content of the residue was estimated by Kjeldahl method and expressed as acid humin N. The combined filtrates and washings were concentrat-

ed to a small bulk *in vacuo* and amino nitrogen in each fraction was determined after van Slyke's method. The amount of each diamino acid N in diamino fraction was estimated by the following methods, viz., arginine by Weber's colorimetric method[22], histidine by Sera's method[41], and cystine by Tompsett's method[42),43] respectively. The purine base N in diamino fraction was also determined by Hitching-Fiske's method[44]. The lysine N was obtained by subtracting arginine-, histidine-, cystine-, and purine base-N from total N in diamino fraction.

The results obtained with a number of algae are shown in Table 16. The figures in the table indicate the amount of nitrogen of each fraction as per cent of total N of the sample. The proportion of each diamino acid N to diamino fraction N is also given in the table.

Results and Discussion

Though the nitrogen distribution in the hydrolysate of whole algae differs depending not only on the part of frond, the season of growth but also on the age of frond within the same species, a clear difference in the nitrogen distribution can be seen among brown, green and red algae.

In all species analyzed, acid humin N values are higher than alkali humin N values. The high value of acid humin N seems due to the presence of a arge amount of carbohydrate in algae.

Amide N values vary according to the species of algae.

In general, diamino N value is higher in green and red algae and low in brown algae. Namely the protein of brown algae may be characterized by the low content of diamino acids and the high content of monoamino acids compared with the proteins of green and red algae. As for the content of each diamino acid both arginine and lysine may be said to constitute the principal amino acids of diamino fraction. The per cent of arginine N in diamino fraction is highest in *Prasiola japonica* (71.6 per cent) and lowest in *Corallina officinalis* (31.3 per cent). The lysine content listed in the table may probably be somewhat higher than actual by reason of the possible existence of other organic bases in the sample which are estimated as lysine by this procedure. Hence it may be regarded that in most algae, especially in green algae, arginine N may be the predominant form of nitrogen in diamino fraction. At any rate both arginine N and lysine N are the principal form of nitrogen in diamino fraction of algae. Among diamino acids, histidine N is far lower compared with arginine N and lysine N.

As mentioned before, the nitrogen distribution varies markedly with the part and the age of frond as well as with the season of their growth in the same species. For instance, in *Undaria pinnatifida*, diamino N value is highest in leaf followed by sporophyll and lowest in stem, histidine exists appreciably in leaf though it is present in minute amounts in sporophyll and stem, and cystine exists considerably in leaf and stem while it is almost lacking in sporophyll. In the case of *Gloiopeltis furcata* the young frond is higher in diamino N and lysine N values, and lower in cystine N value than adult frond, viz., sporophyll and

old one. Thus in this alga the cystine content in crude protein increases while the lysine content decreases with the age of the frond. In general, the cystine content shows a tendency to increase with the age of the found. In *Porphyra tenera*, the cystine content also increases while the lysine content decreases from November to December.

Table 16. Nitrogen Distribution in the Hydrolysate of Whole Algae. Figures indicate the per cent N of each fraction to total N and the ratio of each diamino fraction N to total diamino N. *, fresh water algae.

Chlorophyceae:

Form of Nitrogen	*Prasiola japonica**		*Prasiola japonica**		*Cladophora* sp.*	
Acid Humin N	5.92		5.83		5.70	
Alkali Humin N	1.11		3.54		2.29	
Amide N	9.42		11.27		10.40	
Total Diamino N	27.29	100	26.30	100	19.85	100
Arginine N	14.48	53.1	18.83	71.6	7.63	38.4
Histidine N	2.20	8.1	1.90	7.2	1.39	7.0
Cystine N	0.38	1.4	0.38	1.4	0.42	2.1
Purine Base N	0.55	2.0	0.72	2.8	0.47	2.4
Lysine N	9.68	35.4	4.47	17.0	9.94	50.1
Total Monoamino N	56.26		53.06		61.69	
Date of Collection	Aug. 21		Autumn		May 8	

Form of Nitrogen	*Cladophora* sp.*		*Enteromorpha Linza*		*Enteromorpha compressa*	
Acid Humin N	6.02		6.21		4.78	
Alkali Humin N	4.18		1.16		3.17	
Amide N	12.34		9.67		9.54	
Total Diamino N	21.13	100	20.46	100	27.47	100
Arginine N	8.27	39.2	11.41	55.8	12.23	44.5
Histidine N	3.14	14.9	1.60	7.8	3.31	12.0
Cystine N	0.32	1.5	0.00	0.0	0.38	1.4
Purine Base N	0.61	2.9	0.88	4.3	0.61	2.2
Lysine N	8.79	41.5	6.57	32.1	10.94	39.9
Total Monoamino N	56.33		62.50		55.04	
Date of Collection	Nov. 1		Feb. 20		Feb. 8	

Phaeophyceae:

Form of Nitrogen	*Enteromorpha intestinalis*		*Laminaria* sp.		*Sargassum Ringgoldianum*	
Acid Humin N	6.52		5.12		7.12	
Alkali Humin N	1.26		2.90		1.03	
Amide N	6.91		6.33		15.58	
Total Diamino N	10.84	100	8.40	100	8.50	100
Arginine N	4.17	38.4	4.20	50.0	3.48	40.9
Histidine N	0.72	6.6	0.20	2.4	0.56	6.6
Cystine N	0.00	0.0	0.36	4.2	0.35	4.1
Purine Base N	0.34	3.1	0.40	4.7	0.17	2.0
Lysine N	5.61	51.9	3.24	38.7	3.94	46.4
Total Monoamino N	74.47		77.25		67.77	
Date of Collection	May 16		—		May 9	

Form of Nitrogen	*Undaria pinnatifida* (leaf)		*Undaria pinnatifida* (sporophyll)		*Undaria pinnatifida* (stem)	
Acid Humin N	9.18		5.89		5.21	
Alkali Humin N	1.24		1.27		1.05	
Amide N	9.73		8.27		7.36	
Total Diamino N	11.67	100	8.76	100	7.76	100
Arginine N	4.03	34.5	3.13	35.7	2.72	35.1
Histidine N	0.71	6.1	0.00	0.0	0.00	0.0
Cystine N	1.41	12.2	0.00	0.0	0.85	10.9
Purine Base N	0.51	4.3	0.42	4.8	0.05	0.7
Lysine N	5.01	43.0	5.21	59.5	4.18	53.3
Total Monoamino N	68.18		75.81		78.62	
Date of Collection	Apr. 26		Apr. 26		Apr. 26	

Rhodophyceae:

Form of Nitrogen	*Aeodes lanceolata*		*Chondrus ocellatus*		*Chondrus ocellatus f. canaliculatus*	
Acid Humin N	17.24		11.57		12.95	
Alkali Humin N	1.64		1.60		1.16	
Amide N	6.25		7.56		6.75	
Total Diamino N	25.77	100	21.73	100	15.84	100
Arginine N	13.65	53.0	8.05	37.0	6.32	39.9
Histidine N	0.64	2.5	1.17	5.4	1.65	10.4
Cystine N	0.86	3.3	0.67	3.1	0.67	4.2
Purine Base N	0.45	1.8	0.63	2.9	0.66	4.2
Lysine N	10.13	39.4	11.22	51.6	6.55	41.3
Total Monoamino N	49.10		57.54		63.30	
Date of Collection	May 8		May 9		May 29	

Form of Nitrogen	*Gigartina intermedia*		*Cryptonemia sparsa*		*Grateloupia ligulata*	
Acid Humin N	13.34		14.84		9.42	
Alkali Humin N	1.49		1.05		1.13	
Amide N	7.38		8.14		8.80	
Total Diamino N	17.40	100	22.69	100	17.99	100
Arginine N	6.69	38.4	9.43	41.5	7.70	42.8
Histidine N	1.96	11.3	1.14	5.0	1.07	5.9
Cystine N	0.76	4.4	0.64	2.8	0.73	4.1
Purine Base N	0.33	1.9	0.59	2.6	0.41	2.3
Lysine N	7.68	44.0	10.89	48.1	8.08	44.9
Total Monoamino N	60.39		53.28		62.66	
Date of Collection	May 29		May 9		May 29	

Form of Nitrogen	*Gloiopeltis furcata* (young frond)		*Gloiopertis furcata* (sporophyll)		*Gloiopertis furcata* (old frond)	
Acid Humin N	0.93		3.66		0.45	
Alkali Humin N	1.41		1.16		1.21	
Amide N	8.90		7.45		10.55	
Total Diamino N	23.54	100	20.51	100	19.06	100
Arginine N	9.60	40.8	9.27	45.2	8.22	43.1
Histidine N	1.55	6.6	1.83	8.9	1.77	9.3
Cystine N	0.68	2.9	0.70	3.4	1.33	7.0
Purine Base N	0.46	2.0	0.47	2.3	0.75	4.0
Lysine N	11.25	47.8	8.24	40.2	6.99	36.6
Total Monoamino N	65.22		67.22		68.73	
Date of Collection	May 16		May 16		May 16	

Form of Nitrogen	*Porphyra tenera*		*Porphyra tenera*		*Corallina officinalis*	
Acid Humin N	3.88		3.53		10.54	
Alkali Humin N	1.69		2.17		8.57	
Amide N	9.46		9.69		3.29	
Total Diamino N	26.65	100	23.91	100	20.82	100
Arginine N	12.69	47.6	13.17	55.1	6.51	31.3
Histidine N	2.45	9.2	2.04	8.5	1.04	5.2
Cystine N	0.65	2.5	0.76	3.2	0.94	4.5
Purine Base N	0.71	2.7	0.82	3.4	0.97	4.6
Lysine N	10.15	38.0	7.12	29.8	11.37	54.4
Total Monoamino N	58.32		60.72		56.78	
Date of Collection	Nov. 15		Dec. 8		May 16	

Summary

The distribution of several forms of nitrogen in the hydrolysate of whole algae was estimated by van Slyke's method. The results obtained are as follows:

1. The protein of brown algae is poor in diamino acids and rich in monoamino acids compared with the proteins of green and red algae.

2. The arginine N and lysine N represent the principal form of nitrogen in the diamino fraction of algae.

3. The cystine content in crude proteins of brown and red algae is higher than that of green algae. This amino acid shows a tendency to increase with the age of frond in the same species.

4. The nitrogen distribution differs not only with the part and age of frond but also with the season of growth within the same species.

(5). Amino Acids in the Hydrolysate of Algae

Several investigations have been reported about the amino acids existing in the hydrolysate of algae. Namely, Tadokoro and Ugazin[37] have estimated the cystine content and Mazur and Clarke[36] have determined 16 kinds of amino acids in the crude proteins of *Laminaria*, *Ulva*, *Sargassum*, and *Chondrus* respectively. Tase[34] has demonstrated the existence of following amino acids in the

hydrolysate of *Undaria pinnatifida*, viz., glycine, alanine, gultamic acid, leucine, proline, valine, and aspartic acid. Recently, TAKAGI[45] has studied the amino acids in the hydrolysate of algae by the paper chromatography and shown the existence of cystine, aspartic acid, glutamic acid, glycine, threonine, alanine, valine, and leucine in *Ulva pertusa*; cystine, aspartic acid, glutamic acid, glycine, threonine, alanine, valine, leucine, and proline in *Enteromorpha* sp.; cystine, aspartic acid, glutamic acid, glycine, alanine, valine, leucine, proline, and an amino acid considered as hydroxy glutamic acid in *Enteromorpha Linza*. FUJIKAWA[46], on the other hand, has investigated the nutritive value of the protein of *Porphyra tenera* by testing the increase in weight of rats fed a diet containing *Porphyra* as a sole protein source and concluded that the nutritive value of the protein of *Porphyra* is almost the same as that of the protein of unhulled rice and is somewhat inferior to that of the meat protein of codfish. Accordingly, it seems reasonable to consider that the protein of *Porphyra* contains all of amino acids which are indispensable for the maintenance of the normal growth of rats.

In the present investigation attempt was made to detect, as thoroughly as possible, the amino acids of several algae by the use of paper chromatography.

Method

0.5–1 g of the sample were hydrolysed by boiling with 20 per cent hydrochloric acid for 24 hours under reflux and the hydrolysate was filtered. The filtrate was evaporated to dryness on a boiling water bath to remove hydrochloric acid

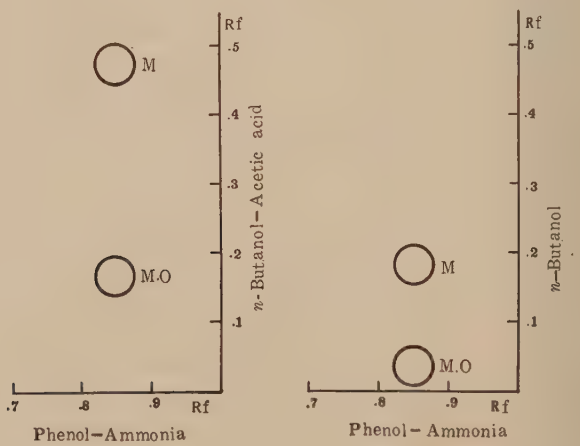

Fig. 12. Standard chromatograms of methionine and methionine sulfoxide.
M, methionine; M.O, methionine sulfoxide.

and the residue was again dissolved in water. The amino acids in this solution were investigated by the paper chromatography. The separation of amino acids on filter paper was made by the two dimensional method. The procedure in detail was the same as described previously. In the present investigation, however, methionine was detected after oxidizing to methionine sulfoxide on a filter paper by treating with hydrogen peroxide and the oxidation product was detected by spraying ninhydrin. The standard chromatograms thus obtained for methionine and methionine sulfoxide are shown in Fig. 12. The results obtained with several algae are given in Table 17.

Results and Discussion

As clearly shown in the table, amino acids other than hydroxyproline, tyrosine, and methionine are found widely in algae.

Mazur and Clarke[36] have already reported the absence of tyrosine in green algae, whereas in the present investigation the existence of this amino acid was demonstrated in *Prasiola japonica* and *Cladophora* sp. Its presence has also been shown in *Porphyra tenera* and *Undaria pinnatifida*. Thus the distribution of tyrosine in algae seems to be comparatively wide.

Table 17. Amino Acids in the Hydrolysate of Algae. *, fresh water algae; +, present; (+), present (probably); ⧺, a large quantity; −, absent.

Name of Algae	Glycine	Alanine	Valine	Leucine & Isoleucine	Phenylalanine	Serine	Threonine	Proline	Hydroxyproline	Tyrosine	Aspartic acid	Glutamic acid	Cystine	Methionine	Arginine	Histidine	Lysine	Tryptophan
Chlorophyceae																		
*Prasiola japonica**	+	⧺	+	+	+	+	+	+	+	+	+	⧺	+	(+)	⧺	+	+	−
Enteromorpha compressa	+	+	+	+	+	+	+	+	−	+	+	+	+	(+)	+	+	+	−
Cladophora sp.*	+	+	+	+	+	+	+	+	+	+	+	+	+	(+)	+	+	+	−
Pheaophyceae																		
Undaria pinnatifida (leaf)	+	⧺	+	+	+	+	+	+	−	+	+	⧺	+	+	+	+	+	−
Undaria pinnatifida (sporophyll)	+	⧺	+	⧺	+	+	+	(+)	−	+	+	⧺	+	+	+	(+)	+	−
Undaria pinnatifida (stem)	+	⧺	+	+	(+)	+	+	(+)	−	−	+	+	+	+	(+)	(+)	(+)	−
Rhodophyceae																		
Porphyra tenera	+	⧺	+	+	+	+	+	+	−	+	+	⧺	+	+	+	+	+	−
Chondrus ocellatus	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	−

The existence of hydroxyproline is limited to two species of green algae, *Prasiola* and *Cladophora*, thus far examined, while proline shows a wide distribution.

Mazur and Clarke[36] and Takagi[47] have reported that methionine does not exist in algae, however, in the present study the presence of this amino acid

was ascertained, though in extremely minute quantities, in *Porphyra tenera*, *Chondrus ocellatus* and in *Undaria pinnatifida*. But the existence of this amino acid in *Enteromorpha compressa*, *Cladophora* sp., and *Prasiola japonica* remains questionable.

Although each diamino acid distributes widely in algae, its quantity is small except arginine in *Prasiola japonica*.

The sort of amino acid in the hydrolysate differs more or less with the parts of frond in the same species. For example, in *Undaria pinnatifida*, some differences in the kind of amino acid can be seen among the leaf, sporophyll and stem.

Among amino acids in the hydrolysate, glutamic acid, alanine and leucine exist in relatively large amount in all algae so far examined. In addition to these amino acids a large quantity of arginine is found in *Prasiola*.

Though the tryptophan has been demonstrated and estimated by MAZUR and CLARKE[36] in the proteins of several marine algae this amino acid could not be detected in the present study. This seems as due to the use of acid hydrolysis for the preparation of the sample solution.

The hydrolysate of *Porphyra tenera* contains many kinds of amino acids that are indispensable for the nutrition of higher animals. Thus the finding of FUJIKAWA[46] that the protein of this alga possesses high nutritive value was corroborated.

(6). Summary of Chapter III

In the present chapter, studies on the amino acids in algae, both free and combined forms, with regard to their kinds, quantities, and seasonal variation have been described. The results can be summarized as follows:

1. *Free Amino Acids*: Of various kinds of amino acids found in the protein-free extract of algae both glutamic acid and alanine are distributed most widely as well as most abundantly. But *Prasiola japonica* contains a large quantity of free arginine in addition to glutamic acid and alanine. As regards diamino acids, arginine is found most commonly in free form and it was isolated and identified as flavianate from the extract of *Prasiola japonica* and *Ulva pertusa* respectively. The content of free arginine in green algae is considerably higher than those in brown and red algae. Thus arginine may be considered to play some important part in the nitrogen metabolism of green algae, especially in *Prasiola*. The seasonal variation of free arginine in *Prasiola japonica* proceeds differently from that in *Porphyra tenera*. Histidine also distributes widely, though in small quantity, and it was isolated as picrate from the extract of *Sargassum Ringgoldianum*. Lysine exists in some of red algae in a free state.

2. *Combined Amino Acids*: The distribution of several forms of nitrogen and the kinds of amino acids in the hydrolysate of whole algae have been investigated by van Slyke's method and by paper partition chromatography.

The proteins of brown algae are characterized by the low content of diamino acids and the high content of monoamino acids compared with those of green and red algae. Arginine and lysine are the principal amino acids in diamino acid fraction and the histidine content is generally low. The cystine content in

brown and red algae is higher than that in green algae. Amino acids other than tyrosine, hydroxyproline and methionine occur widely. The existence of tyrosine was confirmed with *Prasiola* and *Cladophora*. A very small amount of methionine was detected in *Porphyra*, *Chondrus* and *Undaria*.

CHAPTER IV

BETAINES AND CHOLINE OF ALGAE

It is well known that most plants contain one or more kinds of betaines which are derived from amino acids, pyridine carboxylic acids and other various nitrogen compounds. Choline, the constitution of which is closely related to betaine, also distributes widely among plants. Choline occurs in nature both in free and combined forms. Klein and Linser[48] have advanced the hypothesis about the mechanism of the biosynthesis of trigonelline and stachydrine in higher plants. Very little is known, however, about the metabolism and physiology of individual betaine in plant. Recently, dimethylpropiothethine, in which methyl groups combine with sulfur, was found in a red alga, *Polysiophonia fastigiata*, by Chalenger and Simpson[49]. With the homogenate of liver or kindey of rats, guinea pigs, and pigs, dimethylpropiothethine behaved similarly to choline as methyl donor in the methylation of homocysteine[50]. Accordingly it may be considered probable that each betaine might also plays a part in transmethylation reactions.

As regards betaines and choline of algae, Toda[51] has shown the existence of stachydrine in *Porphyra laciniata*, and Zeller[52] has demonstrated the presence of choline in the following algae, i.e., *Enteromorpha* sp., *Fucus vesiculosus*, *Laminaria saccharina*, *Chorda filum*, *Chondrus crispus*, *Cytcclonium purpurescens*, *Delesseria sanguinea*, *Plocanium cocineum*, *Polysiphonia furciculata* and *Rhodomela subfusca* respectively. Contrary to these workers Shirahama[53] could not recognize the presence of choline, glycinebetaine and stachydrine in *Alaria crrasifolia* and *Cytophyllum hakodatensis*.

The present investigations were made on betaines and choline of the marine algae, *Laminaria* sp., *Sargassum Ringgoldianum* and *Ulva pertusa* respectively.

(1). Isolation of Choline and Stachydrine from the Extract of *Laminaria* sp.

Experimental: 2 kg of the sample were extracted twice with 87 per cent alcohol (61. in each) on a boiling water bath each for 6 hours. The extracts were combined and alcohol was distilled off *in vacuo*. The residues were dissolved in water and lead acetate was added and filtered. The excess of lead in the filtrate was removed with sulfuric acid. The filtrate was concentrated to a small bulk *in vacuo* and sulfuric acid was added to 5 per cent. The bases in the extract were precipitated by adding 25 per cent phosphotungstic acid dissolved in 5 per cent sulfuric acid. After standing for 24 hours the precipitate was filtered off on a Büchner funnel and washed with cold 5 per cent sulfuric acid containing a small quantity of phosphotungstic acid. The precipitate was

decomposed with baryta and the excess of baryta was removed with carbon dioxide. The filtrate was concentrated to a small bulk *in vacuo*. The bases in purine and arginine-histidine fractions were removed by means of silver nitrate and baryta according to the procedure of Kutscher-Kossel-Ackermann[15), 29)]. The excess of silver and baryta in the filtrate was removed respectively with hydrochloric acid and sulfuric acid, then the bases in the filtrate (bases in lysine fraction) were again precipitated with phosphotungstic acid. After decomposition of the precipitate with baryta, the solution of the bases was acidified with hydrochloric acid and concentrated to a small bulk *in vacuo*. The residue was taken up in 95 per cent alcohol and alcoholic mercuric chloride solution was added and allowed to stand in a refrigerator for 48 hours. The precipitate was filtered off on a filter paper. In this way the bases in the lysine fraction were divided into soluble and insoluble mercuric derivatives. The insoluble mercuric derivative was dissolved in hot water and decomposed with hydrogen sulfide and filtered. The filtrate was concentrated to a syrup *in vacuo* and kept in a vaccuum desiccator over sulfuric acid for 2 days. The crystal of hydrochloride, which is very hygroscopic and dissolves easily in alcohol, was obtained. By testing the crystal after Klein's method[54), 55)] it was identified with choline hydrochloride, namely the crystalline shapes of periodide and mercuric chloride were perfectly identical with those of choline. The crystal was converted to aurichloride with the yield of 0.0188 g. The aurichloride showed m.p. 249~250°C and the content of gold agreed well with that of choline aurichloride.

Sample	0.0093 g	found	0.0041 g Au	44.1 % Au
Calculated for $C_{15}H_{14}NOClAuCl_3$				44.49% Au

The soluble mercuric fraction was decomposed with hydrogen sulfide, filtered and concentrated to a syrup *in vacuo* and kept in a refrigerator for several days. A small amount of crystal appeared. On examining the crystal after Klein's method[54), 55)] it was found to be identical with stachydrine hydrochloride, the characteristic crystal of stachydrine-bismuth-iodide being obtained. The crystal was converted to aurichloride and a small amount of stachydrineaurichloride, which is yellow in color and rhombic in shape, was obtained. However yield of the aurichloride was so small that further analysis could not be carried out.

(2). Betaines and Choline of *Sargassum Ringgoldianum* and *Ulva pertusa*

Sargassum Ringgoldianum: 2.5 kg of the sample were extracted three times with hot water. The extract was treated in the same way as described *Laminaria*. The bases in the lysine fraction were converted to hydrochloride, evaporated to a syrup and dried in a desiccator. The mixture of the hydrochlorides was extracted with cold absolute alcohol and then with boiling absolute alcohol.

i). *The fraction soluble in cold absolute alcohol*: After removal of alcohol the existence of a small amount of choline was demonstrated.

ii). *The fraction soluble in boiling absolute alcohol*: The bases in this fraction were converted to picrate and 0.01 g was obtained. The shape of the picrate crystal was prismatic and it was difficultly soluble in ethyl alcohol but easily in

water and methyl alcohol; m.p. 200°C. The properties of the picrate resembled to those of trigonelline picrate but the amount of the picrate was too small for further investigation.

Ulva pertusa: 1.3 kg of the ground sample were treated in the same way as described above. The existence of a little amount of choline was demostrated in the lysine fraction.

(3). Distribution of Choline and Stachydrine in Algae

As described in the preceding paragraph, choline seemed to distribute as widely in algae as in higher plants but the distribution of stachydrine remained obscure. Both choline and stachydrine can be recognized qualitatively by the crystalline shape of their salts such as periodide, mercuric chloride or bismuthiodide[54),55)]. The present study was undertaken to elucidate the distribution of these bases in algae.

The ground samples varying in weight from 20 to 100 g were thoroughly extracted with 80 per cent alcohol on a boiling water bath for 3 hours. After distillation of alcohol, the residue was dissolved in water and purified with lead acetate. After the removal of lead from the filtrate by sulfuric acid, it was concentrated *in vacuo* and sulfuric acid was added to 5 per cent. The bases were precipitated with phosphotungstic acid as usual. After standing for 24 hours the precipitate was filtered off on Büchner funnel and decomposed with baryta. The excess of baryta in the filtrate was removed with carbon dioxide. The filtrate was acidified with hydrochloric acid, evaporated to dryness on a boiling water bath and dried in a desiccator over sulfuric acid. The residue was extracted with absolute alcohol on a boiling water bath. After evaporation of alcohol from the extract, the residue was dissolved in a small amount of water.

Table 18. Distribution of Choline and Stachydrine in Algae. +, present; (+), present (probably); −, absent.

Name of Algae	Choline	Stachydrine
Chlorophyceae:		
Codium latum	+	−
Phaeophyceae:		
Sargassum Ringgoldianum	+	−
Sargassum Kjellmanianum	+	−
Myelophycus caespitosus	+	(+)
Laminaria sp.	+	+
Undaria pinnatifida	+	−
Eisenia bicyclis	+	−
Ecklonia cava	+	−
Rhodophyceae:		
Porphyra tenera	+	−
Chondrus ocellatus	+	+
Nemalion pulvinatum	+	(+)

This solution was served for the qualitative test. To the test solution on a slide glass, a drop of the following reagents were added and the crystal appearing on a slide glass was examined under microscope. It was found suitable to use Stanek's iodine solution and saturated mercuric chloride solution in 50 per cent

alcohol for the recognition of choline and potassiumbismuthiodide reagent for stachydrine.

The results obtained are summarized in Table 18. The distribution in algae of choline is wide whereas that of stachydrine is more or less limited.

(4). Discussion and Summary of Chapter IV

In the present investigation, the author has demonstrated stachydrine in *Laminaria* sp. and *Chondrus ocellatus*. This betaine has already been found in *Porphyra laciniata* by TODA[51]. In the present study, however, stachydrine could not be detected in *Porphyra tenera*. According to KLEIN and LINSER[48] stachydrine is formed in plant not only by methylation of proline but also from glutamic acid and arginine. So far as the present investigation is concerned glycinebetaine which occurs widely in higher plants could not be detected in algae. The picrate isolated from *Sargassum Ringgoldianum* resembled to trigonelline picrate, however, ultimate proof awaits further investigation.

Choline was isolated from *Laminaria* sp. It appears to distribute widely in algae.

CHAPTER V
PURINE BASES OF ALGAE

The author has already shown that the content of total purine N of algae is very small and varies markedly with the part of the frond. In *Undaria pinnatifida*, the percentage of total purine N to total N was 0.5 per cent in the frond and 0.05 per cent in the stipe (Table 16).

As regards the free purine bases of algae TAKAGI[9] has shown the existence of adenine in *Porphyra pseudolinearis*. In the present studies the presence of free purine bases was examined with *Laminaria* sp., *Sargassum Ringgoldianum* and *Ulva pertusa*.

(1). Purine Bases of *Laminaria* sp.

Of 4 kg of the powdered sample, 2 kg were extracted with 80 per cent alcohol (51.) on a boiling water bath for 6 hours, and other 2 kg with 2 per cent hydrochloric acid (51.) at room temperature for 24 hours. The alcoholic extract was concentrated *in vacuo* and the acid extract was neutralized to litmus with sodium

Table 19. Nitrogen Content of Adenine Picrate Isolated from *Laminaria* sp. Nitrogen was determined by Kjeldahl method after reducing the picrate with zinc dust and hydrochloric acid.

Sample	N/10 H_2SO_4	N	
		Found	Calculated
0.1193 g	24.70 ml.	29.00%	29.38%

hydroxide and the both extracts were combined. The extract was purified with lead acetate and the excess of lead was removed with sulfuric acid. The filtrate

was concentrated to a small bulk *in vacuo*. Then the bases in the extract were precipitated with phosphotungstic acid as usual. The precipitate was filtered off on a Büchner funnel and decomposed with baryta. After the removal of excess of baryta with carbon dioxide, the filtrate was concentrated *in vacuo* and ammoniacal silver nitrate solution was added. The precipitate formed was filtered off, heated with nitric acid (sp. gr. 1.1) and filtered while it was hot. When the filtrate became cool, the pale yellow precipitate appeared. The precipitate was filtered off, suspended in hot water, and decomposed with hydrogen sulfide and filtered again. The filtrate was concentrated to a small bulk on a boiling water bath, acidified with dilute hydrochloric acid and sodium picrate was added. In this way a small amount of picrate was obtained. The picrate was recrystallized from boiling water. This picrate crystal was needleshaped, hardly soluble in water and melted at 279°C. The nitrogen content of the picrate almost agreed with that of adenine picrate as shown in Table 19. The yield of the picrate was 0.18 g, corresponding to a yield of 0.0016 per cent of free adenine in the sample.

(2). Purine Bases of *Sargassum Ringgoldianum*

2.5 kg of the ground sample were extracted three times with hot water. The extract was treated in the same way as *Laminaria*. Efforts to obtain picrates of purine bases have so far failed, suggesting the absence of free purine bases in this alga.

(3). Purine Bases of *Ulva pertusa*

1.3 kg of the sample were thoroughly extracted with hot water. The purine bases were precipitated with sliver nitrate as descrived above. The precipitate was decomposed with hydrochloric acid, filtered and the filtrate was concentrated on a boiling water both and sodium picrate was added. After standing for 24 hours two sorts of picrates, rhombic and amorphous one, were obtained. Each picrate was recrystallized from boiling water. The yield of rhombic picrate was 0.035 g. It was difficultly soluble in water and on heating in a capillary tube the color changed to black at 205–210°C and melted at 275°C. The nitrogen content of the picrate nearly coincided with hypoxanthine picrate (Table 20)

Table 20. Nitrogen Content of Hypoxanthine Picrate Isolated from *Ulva pertusa*. Nitrogen was estimated by Kjeldahl method after reducing the picrate with zinc dust and hydrochloric acid.

Sample	N/50 H_2SO_4	N	
		Found	Calculated
16.533 mg	14.76 ml.	25.00%	25.60%

The yield of the amorphous picrate was 0.04 g. This picrate was difficultly soluble in water but soluble in ammonia. On heating in a capillary tube the color changed to black at 185°C and melted at 244°C. The nitrogen content of the picrate was 25.63 per cent. The amount of the picrate was so small that further test could not be carried out.

Table 21. Nitrogen Content of Adenine Picrate Isolated from *Ulva pertusa*. Nitrogen was determined by Kjeldahl method after reducing the picrate with zink dust and hydrochloric acid.

Sample	N/50 H_2SO_4	N	
		Found	Calculated
45.716 mg	46.53 ml.	28.50%	29.38%

Upon concentrating the filtrate from hypoxanthine picrate needleshaped picrate was obtained. After recrystallization from boiling water 0.06 g were obtained. This picrate was scarcely soluble in water and melted at 279°C. The nitrogen content of the picrate agreed with adenine picrate as shown in Table 21.

(4). Results and Discussion of Chapter V

In the present investigation the author demonstrated the existence of adenine in *Laminaria* sp. and *Ulva pertusa*, and hypoxanthine in *Ulva*. These results might suggest the wide occurrence of adenine in algae. In view of the well known enzymatic conversion of adenine to hypoxanthine, the occurrence of these two purine bases in the same alga may well be understood.

CHAPTER VI
POLYPEPTIDES OF ALGAE

HAAS, HILL and RUSSEL-WELLS[56), 57), 58)] have found that the protein free extracts of certain brown and red algae gave a marked biuret reaction, the intensity of which diminished with the depth of the habitat of the plants. They concluded that the materials responsible for biuret reaction were octapeptide of glutamic acid and pentapeptide of aspartic acid, which they isolated from *Pelvetia canaliculata* and *Corallina officinalis* respectively. Their polypeptide specimens, however, could not be obtained in crystalline state. Recently, OHIRA[6)] has isolated a crystalline polypeptide from the alcoholic extract of brown alga, *Eisenia bicyclis*, and named it eisenin. He has shown that eisenin is a tripeptide composed of two molecules of glutamic acid and one of alanine. For the constitution of eisenin L(−)pyroglutamyl-L(+)glutaminyl-L(−)alanine has been assigned. The occurrence of polypeptides in algae may be of interest from viewpoint of the nitrogen metabolism.

The present investigation was carried out to make clear the distribution of polypeptide in algae.

Method

Samples varying in weight from 1 to 5 g were extracted thoroughly with 70 per cent alcohol on a boiling water bath for 30 minutes. After evaporation of alcohol the extract was purified with lead acetate and the excess of lead was removed with sulfuric acid. The filtrate was neutralized to pH 6 with dilute

sodium hydroxide and concentrated to a small bulk *in vacuo*.. The biuret reaction was tested with the portion of this concentrated extract. The results are summarized in Table 22.

Results and Discussion

The algae which clearly showed a positive biuret reaction were only three species of brown algae, i.e., *Eisenia bicyctis*, *Ecklonia cava* and *Sargassum Ringgoldianum* thus far observed. Very weak reaction was also given with another brown algae, *Sargassum hemiphyllum*, and *Corallina officinalis* and *Gigartina intermedia* both of which belong to red algae. The polypeptide of *Eisenia bicyclis* may probably be eisenin as demonstrated by Ohira[6]. For the positive reaction of *Ecklonia cava* the same polypeptide may be responsible, since both species are closely related taxonomically. Naturally, however, further investigations are required for the ultimate decision.

Table 22. Distribution of Polypeptide in Algae Observed by the Biuret Reaction of the Extract. *, fresh water alga; +, positive reaction; ±, very weak reaction; −, negative reaction.

Name of Algae	Biuret Reaction
Chlorophyceae	
*Prasiola japonica**	−
Cladophora sp.*	−
Phaeophyceae	
Laminaria sp.	−
Eisenia bicyclis	+
Ecklonia cava	+
Cystophyllum sisymbrioides	−
Sargassum Ringgoldianum	+
Sargassum hemiphyllum	±
Sargassum enerve	−
Ishige Okamurai	−
Rhodophyceae	
Chondrus ocellatus	−
Grateloupia elliptica	−
Corallina officinalis	±
Gigartina intermedia	±

GENERAL SUMMARY

The present investigation has been performed for the purpose of making clear the fundamental features about nitrogen compounds which are considered as physiologically important to algae. The results can be summarized as follows:

(1). *Forms of nitrogen in algae and their content.*

The greater part of nitrogen of algae is composed of protein N and besides this a relatively little quantity of non-protein N exists. In general, the total N content is relatively high in green algae and low in brown algae. In red algae it is high in Bangiales and low in Florideae. The non-protein N content varies

according to the species. Certain species such as *Prasiola japonica*, *Laminaria* sp., and *Sargassum Ringgoldianum* show considerably high value of non-protein N. The total-, protein- and non-protein-N contents vary more or less considerably with the season of growth, the parts as well as with the age of the frond in the same species. The mode of the seasonal variation of these nitrogen compounds of *Porphyra tenera* and of *Higikia fusiformis* differs considerably from that of *Prasiola japonica*. Namely in *Porphyra* and *Higikia*, the seasonal variation of total N content is due to the change of protein N content, while in *Prasiola* this is, for the most part, attributed to non-protein N. This phenomenon seems to be characteristic of the nitrogen metabolism of this alga.

(2). *Forms of nitrogen in non-protein fraction and their content.*

In general, amino N is the principal form of nitrogen in the non-protein nitrogen fraction of algae. In some species of green algae, especially in *Prasiola japonica*, however, major part of nitrogen in non-protein fraction is represented by basic N which is followed by amino N. Thus, in this alga, free diamino acids are the main nitrogen compounds in the non-protein fraction, whereas in most brown and red algae free monoamino acids may be regarded as the principal ones where basic N contents are far lower than non-basic N contents. Other forms of nitrogen such as volatile N and amide N are poor. Each form of nitrogen in non-protein fraction undergoes a marked seasonal variation. In *Porphyra*, the seasonal variation of non-protein N may be represented mainly by the seasonal variation of free monoamino acids, whereas in *Prasiola*, it is due to that of free diamino acids.

(3). *Free Amino acids*

Many kinds of the amino acids exist in a free state, among which both glutamic acid and alanine are important not only in the width of distribution but also in quantity. Aspartic acid and cystine show a next wide distribution. The occurrence of other amino acids varies from species to species but the existence of free methionine seems doubtful. In *Prasiola japonica*, however, a large quantity of arginine exists in addition to glutamic acid and alanine. Among diamino acids arginine is the most common in occurrence which is followed by histidine. Lysine seems to be present in red algae. The free arginine content of green algae is far higher than that of brown and red algae and it varies markedly with the season. In *Prasiola japonica* the content of free arginine attains its maximum (2.6 per cent on dry basis) in winter when the sexual reproduction of this alga takes place. As regards amide compounds, the existence of asparagine was demonstrated in some of green algae whereas glutamine could not be detected with certainty.

(4). *Amino acid composition of protein.*

The protein of brown algae is relatively poor in diamino acids and rich in monoamino acids as compared with those of green and red algae. The amino acids other than hydroxyproline, tyrosine and methionine are the most common constituents of the proteins of algae. Of diamino acids both arginine and lysine

are the major amino acids. Histidine is also widely distributed but its content is lower than the above two diamino acids. Methionine was found to exist in *Porphyra tenera*, *Chondrus ocellatus* and *Undaria pinnatifida*, however, in minute quantity. The distribution of tyrosine as a constituent of algal protein, which has been missed in green algae, appears to be comparatively wide. The cystine content of the proteins of brown and red algae is higher than that of green algae. The amino acid composition of protein varies more or less considerably with the age as well as with the part of frond in the same species, viz., in *Gloiopeltis furcata* the protein of young frond is higher in lysine and lower in cystine than that of the old one.

(5). *Betaines and allied compounds.*

The existence of stachydrine was demonstrated in *Laminaria* sp., and in certain other species. Other betaines could not be identified with certainty.

Choline was isolated as aurichloride from *Laminaria* sp. It appears to occur widely in algae as in higher plants.

(6). *Purine bases.*

The existence of adenine was demonstrated in *Laminaria* sp., and *Ulva pertusa*. The presence of hypoxanthine was confirmed in *Ulva pertusa*, however, it is uncertain whether this base exists as such in living alga or it is formed secondarily from adenine after harvest by the action of an enzyme, purine deaminase.

(7). *Polypeptides.*

The protein-free extracts of certain brown algae such as *Eisenia bicyclis*, *Ecklonia cava* and *Sargassum Ringgoldianum* indicate a positive biuret reaction. This may be taken to indicate the presence of some lower, alcohol soluble polypeptides in these algae.

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BIOCHEMICAL STUDIES ON THE VITAMIN A IN FISH VISCERA
III. THE OXIDATION OF VITAMIN A IN FISH LIVER OILS

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In the previous report[1] authors showed that the loss of vitamin A was markedly affected by the temperature during the storage of fish livers, and the destruction of vitamin A might be caused by the catalytic action of a certain enzyme system.

Later it was observed that vitamin A was rapidly oxidized a great portion of its content, and this oxidation mechanism is supposed to be similar to the case of the oxidation by air or oxygen.

In 1932, André and Hou[2] discovered a specific enzyme in soy-bean, so-called lipoxidase which attacks the higher unsaturated fatty acids with several double bonds. The lipoxidase occurs in plant seeds, particularly in legumes[3], in potato tubers[4], and to some extent in animal tissues[5,6]. The last finding has been questioned since the ordinary iron porphyrins catalyze the oxidation of multiple unsaturated fatty acids without an enzyme system[7]. Haurowitz *et al.*[8] observed that pure crystalline hemoglobin which occurs in animal tissues in large quantities, acts as a good catalyst in the direct oxidation of fatty acids by oxygen. This is even true of hemin which contains no protein. Since the porphyrin ring itself undergoes peroxidative destruction through this reaction, it is still in doubt that the lipoxidase occurs in animal tissues. However linoleic acid-cholesterol esters are apparently protected against oxidation by blood hemoglobin, and therefore it may be the loss caused by the enzymatic oxidation of fats in animal tissues.

Lipoxidase was isolated by Cosby and Sumner[9], and Theorell, Holman, and Åkeson[10] were able to obtain crystallized lipoxidase.

It is of interest to determine whether this lipoxidase posseses or not the oxidizing activity for vitamin A which is contained abundantly in fish liver oils. Frey[11], Hauge and Aitkenhead[12], and Hauge[13] attributed the loss of vitamin A activity in dried alfalfa to an enzymic factor, later shown to be lipoxidase. Wilbur et al.[14] found that incorporation of soy-bean in diet of diary cows led to the decrease in vitamin A activity of the butter produced.

Some studies, however, on the oxidizing enzyme of vitamin A have been made on the fish tissues; Brocklesby[15] observed the enzymatic oxidation of vitamin A in salmon liver, Banks[16,17] investigated a lipoxidase-like enzyme in herring muscle, and Khan[18] in the dark muscle of herring.

Authors observed an enzyme action on vitamin A in some fish livers, and attempted to determine the identity of the lipoxidase and an oxidizing enzyme in fish tissues and the oxidation mechanism of unsaturated fatty acids by the enzyme.

EXPERIMENTAL

I. Materials.

(*1*) *Acetone-dried liver powder.*

Fresh mackerel liver ground three times in a chopper was added to five volumes of ice-cold acetone. The mixture was strongly shaken and allowed to stand in the cold place for about three hours and filtered by cotton. The residue remained on filter was further treated with three volumes of ice-cold acetone, filtered by suction and added to ice-cold ether, treating above mentioned, and dried quickly in air spreading over the filter paper. The dried powder was kept in a vacuum decicator in the refregerator.

(*2*) *Acetone-dried soy-bean powder.*

Ground 150 g of soy-bean which were stored carefully and dried in a vacuum dryer, passing a grinder several times, and powdered by usual process treating with 900 cc of acetone and 200 cc of ether. Sample was used as a fine powder through a 150-mesh sieve, and should be stored in a ice-box (refregerator) to prevent the loss of the enzyme activity.

(*3*) *Liver oil.*

Common tuna liver oil refined with 0.1% alkali water solution, washing three times with hot water and dried dehydrating with anhydrous Na_2SO_4, was used. 1 g of this refined tuna liver oil contained 80,000 International Units of vitamin A.

II. Methods.

(*1*) *Preparation of reaction mixture.*

5 g of acetone-dried liver powder was extracted with 15 cc of phosphate buffer solution (pH 5.4) for 1 hour in cold. The extract, squeezing through linnen (or muslin), was centrifuged at 3,000 rev./min. The clear centrifuger was dialysed overnight at 5°C against 4 liters of distilled water. The dialysate was determined the oxidizing activity in the presence of such emulsifyers as albumin, arabia gum, sodium arginate, carboxy methyl cellulose, etc.

(*2*) *Estimation of the enzyme activity.*

Added 0.2 g of tuna liver oil and 1.5 g of carboxy methyl cellulose (commercial name, "CMC" or "Neo Colloid") to 30 cc of enzyme solution, and mixed in the cold to form a perfect emulsion. After incubation at 37°C for 30 minutes, added to this reaction mixture, 5 cc of methyl alchohol in order to stop the enzyme action. The tuna liver oil in the reaction mixture which was oxidized by the enzyme was separated from the mixture by the extraction with ether, and estimated the vitamin A contents by the colorimetric and spectroscopic methods.

The colorimetric method was the one using a tintometer by Carr-Price reaction, and spectroscopic method was absorption spectra method using the Beckman DU quartz spectrophotometer. The enzyme activity was indicated in the loss of vitamin A.

RESULTS

1. Effect of vitamin A concentration.

Experiments were carried to observe the influence of vitamin A concentration to the enzyme activity. In the case of lower concentration, 1 g of the reaction mixture contained about 500 I.U. of vitamin A, and in higher concentration contained 6,800 I.U. The results are shown in Table 1.

Table 1. Effect of vitamin A concentration.
(* gL means g of liver.)

Time (hr.)	0		2				24			
Vitamin A / Temp. °C	Content		Content		Loss		Content		Loss	
	I.U.	%	I.U.	%	I.U./gL*	%	I.U.	%	I.U./gL	%
37	500	100	335.1	67.02	33.0	6.6	135.0	27.00	71.0	14.2
60	500	100	325.0	65.00	33.0	6.6	—	—	—	—
37	6800	100	5960.0	87.65	168.0	2.5	—	—	—	—
60	6800	100	4370.0	64.26	494.0	7.3	—	—	—	—

The oxidation coefficient of vitamin A at 37°C decreased corresponding to the increase of vitamin A concentration. On the contrary, the oxidizing activity at 60°C was not affected by the amounts of vitamin A under a certain condition, but it is shown that there is a considerable effect of temperature.

2. Effect of substrate (oil) concentration.

Prepared two reaction mixtures in which one contained 58.6% of tuna liver oil (a), and another 34.7% (b). These mixtures were prepared as the emulsions from which liver oil were inhibited the separation during the incubation, using a homogenizer with the acetone-dried mackerel liver powder, buffer solution, and emulsifyer. Results are shown in Table 2.

If samples contained liver oil of more than 50% in the reaction mixture, the enzyme activity did not increase but decrease rather slightly corresponding to the reaction time. On the contrary, the increase of activity at higher temperature depends on the ordinary chemical reaction.

In the case of (b), the liver oil content of 34.7%, the loss of vitamin A increased always at each temperature. The oxidizing activity of vitamin A per g of the acetone-dried liver powder were 1,252 and 1,870 in (a) and (b) at the reaction of 4 hours, namely the ratio of (a) and (b) is about 1.5.

3, The type of the enzyme.

Experiments were made to determine the type of the oxidizing enzyme of vitamin A in mackerel liver though it seemed to be a lyo-enzyme. Ground 10 g of the acetone-dried liver powder with 60 cc of phosphate buffer solution and 0.2 cc of chloroform, and incubated the mixture stiring sometimes at 20°C for 1 hour. By the centrifuger the mixture was fractionated into the soluble and insoluble portions. These two fractions were determined their enzyme activities by the methods above mentioned. From this experiment, it was shown that the

type of the oxidizing enzyme of vitamin A in fish livers might be a lyo-enzyme (Table 3).

Table 2. Effect of substrate concentration.
* In C.L.O.Units.

Temp. °C		21		37		60	
Concentration of liver oil %	Time (hr.)	Vitamin A		Vitamin A		Vitamin A	
		$E^{1\%}_{1\,cm}$	%	$E^{1\%}_{1\,cm}$	%	$E^{1\%}_{1\,cm}$	%
58.6	0			45.2	100	45.2	100
	2			42.1	93.1	40.7	90.0
	4			41.5	91.8	36.8	81.4
	6			41.3	91.2	36.4	80.5
	10			41.1	91.0	34.5	76.3
	24			40.5	89.6	33.4	73.9
Control	0			45.2	100	45.2	100
	2			44.8	99.1	44.4	98.2
	4			44.0	97.3	—	—
	6			43.8	97.0	40.9	90.5
	10			43.2	95.6	38.8	86.0
	24			42.8	94.7	—	—
34.7	0	257.0*	100	257.0*	100	257.0*	100
	2			224.0	87.3	222.0	86.3
	4			214.5	83.5	213.0	82.9
	6			198.3	77.2	190.5	74.2
	10			195.0	75.9	180.0	70.0
	24	160.5	62.5	157.0	61.1	146.1	51.9

Table 3. The type of the enzyme.

Time (hr.)		0		2	
Fraction	Temp. °C	Vitamin A		Vitamin A	
		I.U.	%	I.U.	%
Filtrate	37	2,000	100	292	14.60
	60	2,000	100	684	34.20
Residue	37	3,080	100	2,728	88.57
	60	3,080	100	2,688	87.27
Control	37	1,984	100	1,956	98.50
	60	1,984	100	1,936	97.70

4. Effects of temperature and pH.

As the purity of enzyme affects the oxidizing activity for vitamin A which is always variable by temperature and pH, the use of crude enzyme preparations might not give the available result. Since we could make the approximate value through the last investigation, experiments were carried as follows on these problems.

(a) Effect of temperature.

Each results at 17°C, 37°C and 60°C showed that the enzyme activity

increased rapidly for the primary 3–5 hours and then vitamin A was slowly oxidized. But at the lower temperature (0°C) the enzyme activity was almost negligible at the early stage, while the 40-%-loss of vitamin A 60°C for 35 hours (Table 4, Figure 1).

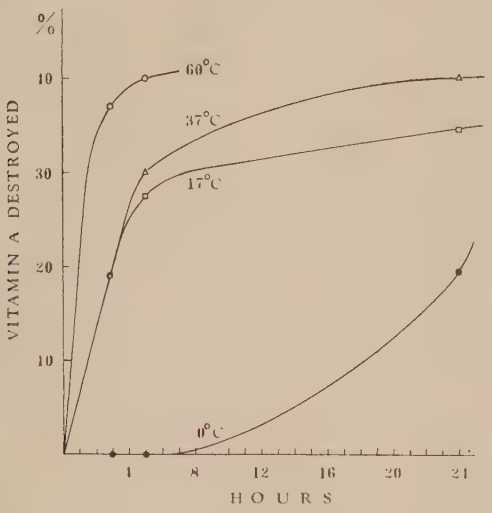

Fig. 1. Effect of temperature on the enzyme activity.

Table 4. Effect of temperature.

Time (hr.)	0		3		5		24	
Temperature °C	V.-A		V.-A		V.-A		V.-A	
	CLOU	%	CLOU	%	CLOU	%	CLOU	%
0	5.9	100	5.9	100	5.9	100	5.0	80.7
17	5.9	100	4.8	81.3	4.3	72.9	3.9	66.1
37	5.9	100	4.8	81.3	4.1	69.5	3.5	60.0
60	5.9	100	3.7	62.8	3.5	60.0	—	—
Control	5.9	100	5.9	100	5.8	99.0	5.8	99.0

From these results it would be considered that the enzyme activity is more inhibitable at lower temperature.

(b) Effect of pH.

As shown in Table 5 and Figure 2, the optimum pH of oxidizing enzyme of mackerel liver was about pH 6–7, approximately pH 6.5, and the optimum pH of the lipoxidase of soy-bean, but this question will be resolved through the comming experiments.

Table 5. Effect of pH.
(Incubation for 20 hours)

Enzyme	pH	E $\frac{1\%}{1\,cm}$	Ratio $300/328_{m\mu}$	Ratio $350/328_{m\mu}$	Vitamin A oxidized	
					E $\frac{1\%}{1\,cm}$	Correct %
Mackerel liver	5.4	21.30	94.5	51.4	19.80	38.18
	6.5	9.70	151.8	44.8	31.40	67.26
	8.0	22.50	91.2	54.1	18.60	42.32
	9.1	25.00	84.1	54.8	7.50	15.37
	11.0	28.40	80.0	55.2	2.70	3.55
Soy-bean	5.4	33.27	78.8	54.9	7.83	9.90
	6.5	30.80	74.4	55.0	10.30	17.38
	8.0	25.90	81.4	52.7	15.20	34.30
	9.1	31.50	69.6	55.1	9.60	20.33
	11.0	39.40	70.0	55.7	1.70	1.18
None (Control)	5.4	37.45	69.5	55.6	3.65	—
	6.5	38.15	69.3	56.0	2.95	—
	8.0	40.40	66.3	56.2	0.70	—
	9.1	40.10	66.6	56.2	1.00	—
	11.0	39.90	66.0	56.3	1.20	—
Original oil		41.10	67.7	56.0	0	—

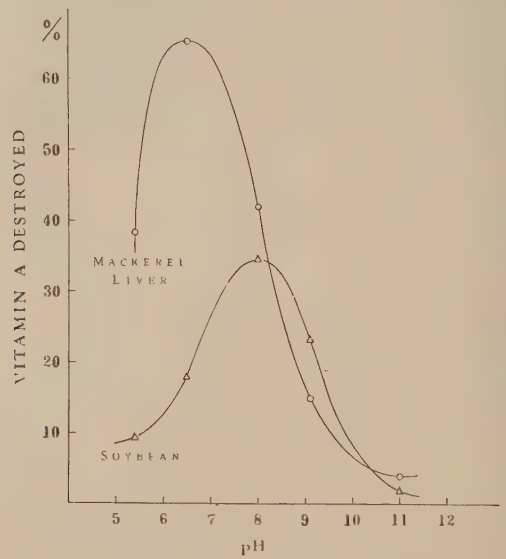

Fig. 2. Effect of pH on the enzyme activity. (Incubation for 20 hours.)

5. Enzyme activity of fish livers free from vitamin A.

An experiment was carried out concerning with the enzyme activity of fish livers contain less or no vitamin A. As such fish, a sea perch, *Lateolabrax japonicus* (*T.* et *S.*), was chosen which were caught by us in Tateyama Bay

Table 6. Enzyme activity of perch liver. * Emulsion of 27 cc of mackerel liver extract (corresponding to 5 g of liver powder), 7 g of tuna liver oil and 0.4 g of emulsifyer. ** Emulsion of 27 cc of mackerel liver extract, 23 cc of perch liver extract (15 g of fresh liver), 7 g of liver oil, and 0.7 g of emulsifyer. *** Emulsion of 23 cc of perch liver, 5 g of liver oil, and 0.7 g of emulsifyer. All were incubated at 37°C for 24 hours.

Wave length ($m\mu$) \ Sample No.	1*	2**	3***	Control
300	28.6	23.3	24.3	29.6
310	35.9	26.2	28.3	37.8
320	40.5	27.5	30.3	42.9
325	42.0	28.0	31.2	45.0
328	42.0	27.7	31.0	45.2
334	38.7	24.8	28.3	41.1
350	23.9	14.8	20.0	25.2
Ratio 300/328 $m\mu$	68.2	84.4	78.4	65.5
Ratio 350/328 $m\mu$	57.0	53.5	54.7	55.8

on May 30th, 1953. The fresh livers were removed immediately from the fish bodies, and used without drying. Results shown in Table 6 and Figure 3 point out that an abundance of lipoxidase-like enzyme occurs even in the liver free from vitamin A.

6. Properties of the oils oxidized by the enzyme.

(a) The absorption spectra of vitamin A in the oxidized liver oils.

In the previous report[1] it has been shown that the autolysed liver oil varied strongly the optical density of ultraviolet light in accordance with the promotion of oxidation, and the absorption spectrum curve would be flattened at shorter wave length than 300 mμ.

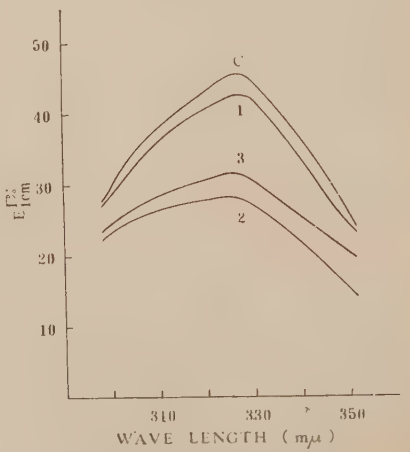

Fig. 3. Enzyme activity of perch liver. 1, 2, 3, and C are identical to the sample No. in Table 6.

As shown in Figure 4, the absorption spectrum curves of vitamin A in liver oil were made to illustrate the enzymatic oxidation by a lipoxidase of soy-bean and a lipoxidase-like enzyme of fish livers. It was assumed that the vitamin A oxidized slightly made a similar absorption spectrum curve having a peak at 328 mμ. as the one of original liver oil, but in the promotion of oxidation for a long time, the curve was so much flattened as having a peak in shorter wave length than 300 mμ.

It is not now obvious whether these curves were resulted from the oxidation products of vitamin A or of the higher unsaturated fatty acids.

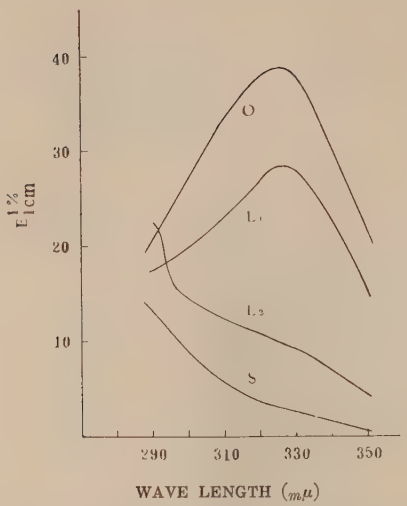

Fig. 4. Absorption spectra of vitamin A in the oxidized liver oils. Original oil is shown in "O", oxidized oils by liver extract in "L_1" and "L_2", and oxidized oil by soybean in "S".

(b) Fish liver oil.

It is of great interest to determine whether the oxidizing enzyme of liver is also able to oxidize the fatty acids as vitamin A. The previous report has shown that the liver oil extracted from the autolysed mackerel liver had no change in its properties, especially the iodine value, against the destruction of vitamin A. Tables 7 and 8 show evidently that there were no differences in its properties, either before or after the reaction of enzyme; the iodine value and Kreis reaction test, except Schiff's reaction.

(c) Linseed oil.

Since the fatty acids of liver oil were seemed to be not oxidized by an enzyme action in order to presence of vitamin A which acts as an antioxidant in fish liver, we attempted to investigate the oxidation system for linseed oil free from vitamin A.

Chemical changes on oil were observed by estimating the iodine value, acid value and peroxide value except vitamin A. Peroxide value was determined

Table 7. Properties of oxidized liver oil.

Time (hr.)	0			2			4		
Sample	Vitamin A		Iodine value	Vitamin A		Iodine value	Vitamin A		Iodine value
	CLOU	%		CLOU	%		CLOU	%	
No. 1	150.3	100	122.8	113.2	75.3	123.0	93.9	62.5	121.8
No. 2	150.3	100	123.0	110.5	73.5	122.5	94.5	62.9	123.2
No. 3	150.3	100	123.1	109.3	72.7	122.0	95.3	62.8	124.0
Control	150.3	100	122.5	150.0	100	124.0	148.0	98.5	122.5

Table 8. Properties of oxidized liver oil.

Time (hr.)	0		2		4		6		10		24	
Vitamin A content	CLOU	%	CLOU	%	CLOU	%	CLOU	%	CLOU	%	CLOU	%
	257	100	230	89.5	230	89.5	210	81.7	210	81.7	185	72.0
Schiff's reaction	+		+		+		+		+		+	
Krei's reaction	−		−		−		−		−		−	

by the Lea's method[19].

Results obtained from the oxidation of linseed oil were shown in Table 9 in addition to tuna liver oil. The iodine value was not affected by the enzyme action and the acid value increased in the course of the enzymatic oxidation, but the increase of acid value would be perhaps indebted to the lipase which is present in the enzyme preparations. The oil oxidized enzymatically had much lower peroxide value than the control without enzyme action.

It is obvious that the fatty acids in linseed oil were also not attacked by an lipoxidase-like enzyme of fish livers, similarly as in tuna liver oil.

Table 9. Properties of oxidized oils.
* cc of $Na_2S_2O_3$ (N/200) solution.

Enzyme	Time (hr.)	Tuna liver oil			Linseed oil		
		Peroxide value*	Iodine value	Acid value	Peroxide value	Iodine value	Acid value
Mackerel liver	24	6.5	156.8	1.7	1.1	226.1	4.5
	48	6.9	150.5	1.5	0.4	212.8	8.1
Control (pH 6.5)	24	9.2	155.1	0.5	5.3	226.5	0.4
	48	11.8	150.0	0.7	6.6	216.5	0.4
Soy-bean	24	9.9	156.1	0.8	6.0	211.7	1.9
	48	5.0	155.7	0.9	7.0	209.2	1.5
Control (pH 9.0)	24	7.2	155.1	0.7	10.5	209.8	0.6
	48	8.7	156.3	1.2	9.3	211.1	0.5
Original oil	—	2.7	156.8	0.4	2.7	226.5	0.5

SUMMARY

1. Vitamin A in liver oil was markedly destroyed by a certain oxidizing enzyme which occurs in some fish livers.

2. Vitamin A oxidized by the lipoxidase of soy-bean and the lipoxidase-like enzyme of fish liver gave similar absorption spectrum curves which had lower peak at 328 mμ, but it was assumed that the curve was flattened in accordance with the promotion of oxidation.

3. The enzyme was found not only in mackerel liver but also abundantly in perch liver which contains entirely no vitamin A.

4. The enzyme was a lyo-form and possessed a special thermal property as having more activity at 60°C.

5. The higher fatty acids in liver oil and linseed oil were less oxidized in the states of glyceride by this enzyme.

6. A large quantity of liver oil inhibited the enzyme activity at room temperature, but at higher temperature the enzyme activity began to develop by breaking down of inhibitants caused by the higher temperature.

7. The enzyme had many similar properties to the soy-bean lipoxidase,

but it might be not identical in all respects for both enzymes, since the optimum temperature and pH of the liver enzyme differed from the one of soy-bean lipoxidase.

Concluding this report, we wish to express our thanks to Mr. M. Suda, a member of the Measurement Institute of Wilbur Ellis Co. Ltd., for measurement of absorption spectrum, and to the financial aid given by the Ministry of Education.

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ON THE INFLUENCE OF γ-RAY RADIATION ON THE AQUATIC ANIMALS

I. ON THE INFLUENCE IN THE EARLY DEVELOPMENT OF GOLD-FISH (*CARASSIUS AURATUS* L.)

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(Received Sept. 1, 1954)

It has been stated by many investigators that the radioactivity has a bad influence upon the animals. From the standpoint of the aquatic field, the influence of radioactivity on the marine products[1,2] is a serious matter, as it acts closely on the aquatic resauces.

Up to now the studies concerning the influence of radioactivity on the animals have been carried out by various means, such as the observation of the influence on the development by the use of uranium nitrate[3], the biological test in the impediments by the X-rays[4,5,6], other ionizing rays[7,8,9] and radioactivity material[10,11], and the application of radioactive rays (mainly X-rays) to make multations in heredity. Systematic experiments on the influence of Co^{60} (γ-rays) radiation upon the aquatic animals have not been carried out. Therefore the authors attempt preliminary studies on the influence of Co^{60} (γ-rays) on the gold-fish under the early development. Here, authors thank Dr. H. Yamashita, and the members of the Keiō Univ. Hospital, whose troubles were taken about the exposure of Co^{60}. We are also indebted to the members of the Tokyō Fisheries Experimental Station and the Mizumoto branch, for their kindness in sending the samples.

Experimental

(1) Experimental place.

1. Gathering place of samples.
   Exp. I. The Tokyō Fisheries Experimental Station (Haneda, Tokyō).
   Exp. II. The Tokyō Fisheries Experimental Station (Mizumoto branch).
2. Irradiation place.
   The Keiō University Hospital (Sinanomachi, Tokyō).
3. Culture place
   The Tokyō University of Fisheries (Yokosuka).

(2) Period.

Exp. I. From 28 May to 8 June, 1954.
Exp. II. From 24 June to 2 July, 1954.

(3) Materials and method.

The experiments were tried two times in the same way. The materials of eggs were of the same kind, *Ryūkin* (*Carassius auratus* L.), both in the first and second experiment. On the first experiment, the eggs spawned on May 27,

1954, were used at the Tokyō fisheries experimental station and on the second experiment those spawned on June 23, 1954 at the Mizumoto branch of Tokyō fisheries experiment station were used. Six samples of eggs were prepared in an ordinaly test tube (d, $1.7 \times l$, 16.5 cm.) with 20 ml. of water, as they adhered to water weeds. One of them was preserved as an unexposed and the other five were exposed to γ-rays radiation.

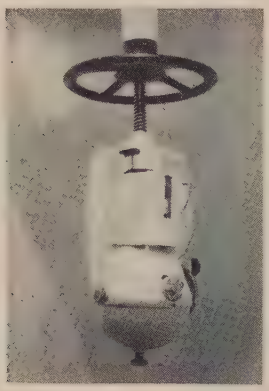

Fig. 1. The apparatus for projection of Co^{60} 41C. (γ-rays).

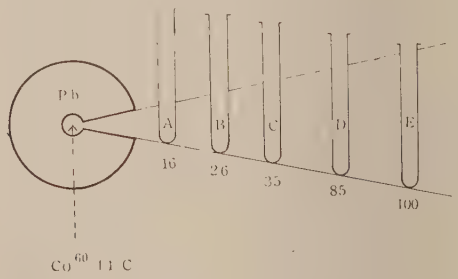

Fig. 2. The condition of γ-ray projection. Distance from Co^{60} 41 C.; A, 16 cm; B, 26 cm; C, 35 cm.; D, 85 cm.; E, 100 cm.

The irradiated apparatus and the condition are shown in Fig. 1 and 2.

Before the studies the influence of each irradiated quantities of γ-ray upon the development of the eggs could not be estimated, so five kinds of irradiation doses were preliminary selected. The various doses were obtained by varying

Table 1. The quantity of projected gamma-rays. Dist, distance from Co^{60} 41 C.; h, hours projected of γ-rays; r., quantity of projected γ-rays; I, the first experiment (projected from p.m. 4.35, 28 May to a.m. 8.35 of the next day); II, the second experiment (projected from p.m. 6.00, 24 June to a.m. 8.30 of the next day.

Sample	Dist	I		II	
		h.	r	h.	r
A	16 cm.	16	31,500	14.5	28,500
B	26	〃	11,900	〃	10,800
C	35	〃	6,600	〃	6,000
D	85	〃	1,100	〃	1,000
E	100	〃	800	〃	730

the duration of irradiation and the distance from the source. Table 1, shows the irradiation doses of five samples and the figures of doses in this table were temporarily calculated as Co^{60} 1 C. $\fallingdotseq 1.2r$/h/m.

During radiation, the "control group" was left at the same condition in

the other room in order to avoid the influence of radioactivity.

After irradiation each sample was brought to the laboratory, and put into a petri dish (*d.* 20×*h.* 5.5 cm.) with the pond water, then the development of them was observed day by day.

During this period it was reported that the rain contained some radioactivity in the country, so radioactivity of the pond water was measured every day by G.M. counter, but it was 25±5 C./100 ml. each time.

The temperature of breeding water was from 16.0°C to 20.5°C (mean value was 18.9°C) on the first experiment and on the second was from 18.0°C to 22.0°C (mean value 19.9°C). And the oxygen quantity contained in this water was good enough to breed to gold-fish eggs.

(4) Results and observation.

(A) Experiment I.

All eggs were hatched from evening of 2 June (4 days after irradiation) to the next morning except "16 cm. group". The hatch rate and the number of the death were shown in Table 2 and 3.

Table 2. Hatching rate and mortality of gold-fish (Exp. I). d, distance from Co^{60} 41 C. source; days, number of days after irradiation; H, number of hatch; H. rate, hatch rate.

d	Days / No.	Mortal						Hatch out	
		0	1	2	3	5	Total	H	H rate
16 cm.	116	6	4	3	7	96	116	0	0%
26	198	9	4	12	10	63	97	101	51.0
35	114	4	8	6	4	4	26	88	77.2
85	156	6	13	15	11	5	50	106	67.9
100	242	8	18	17	5	1	49	193	79.8
Control	272	4	17	10	2	1	34	238	80.5

Table 3. The mortality of the young (Exp. I). d, distance from Co^{60} 41 C. source; No, total number of the young; days, number of days after irradiation.

d	Days / No.	5	7	8	9	10	11	Existence
16 cm.	0	— %	—	—	—	—	—	— %
26	101	100.0	—	—	—	—	—	—
35	88	51.1	100.0	—	—	—	—	—
85	106	51.9	67.9	77.8	77.8	79.6	81.4	18.6
100	193	10.9	11.4	11.4	11.4	11.4	11.4	88.6
Control	238	0	0.4	0.4	0.4	0.4	0.4	99.6

The "16 cm. group" was all dead just before they were hatched.

Five days after irradiation the "26 cm. group" was hatched out, but immediately after they were dead, and the "35 cm. group" hatched out next day.

Thus the more the intensity of irradiation increased the more hatch rate decreased in its number.

In the first experiment the following deformities were observed, the curvature of the vertebrae and the tails and the hypertrophy of the yolk-sac and the pericardium. These deformities are shown microphotographically in the Plate VII, 1–3.

So the authors to know how the intensity of γ-rays infected the early development of gold-fish eggs, but in the first experiments the authors could not have the time enough to spare for the detailed microscopic observation.

(B) The second experiment.

From the previous experiment, it could be seen that the range of the irradiated doses from about 800 r to 30,000 r was fit to observe the infection of gold-fish eggs, therefore at the second experiment the same irradiation doses as the first experiment was selected.

Except "16 cm. group" the eggs hatched out from the evening of 28 June (three days after irradiation) to the next morning.

These hatch rates, abnormal hatch rate, and mortality of the young are

Table 4. Hatch rate and mortality of gold-fish eggs, exposed to γ-ray radiation (Exp. II). d, distance from Co^{60} 41 C. source; No., total number of eggs; N.H., number of normally hatch; A.H., number of abnormal hatch; A, major deformity; B, minor deformity.

d.	Mortal							Hatch out				
	Days / No.	0	1	2	3	4	Total	N.H.	A.H.	Total	N.H. rate	A.H. rate
16 cm.	30	2	1	0	6	21	30	0	—	—	—%	— %
26	65	9	5	3	11	9	37	7	14	21	22.2	58.3 (A.50.0 B.8.3)
35	84	15	7	2	2	3	29	24	30	54	28.6	55.6 (A.14.8 B.40.8)
85	65	10	7	4	0	0	21	36	8	44	55.4	18.2 (A. 4.6 B.13.6)
100	54	12	6	3	0	0	21	30	3	33	55.6	9.1 (A. 0 B. 9.1)
Control	42	3	1	0	0	0	4	38	0	38	90.5	0

Table 5. The mortality of the young (Exp. II). d, distance from Co^{60} 41 C. source; No., total number of the young; days, days after γ-radiation.

d.	Days / No.	5	6	7	Existence
16	0	— %	—	—	0 %
26	21	81.0	100.0	—	—
35	54	1.9	5.7	27.9	32.1
85	44	0	11.4	11.4	88.6
100	33	0	0	0	100.0
Control	38	0	0	0	100.0

shown in Table 4 and 5.

Within three days after irradiation, even the "16 cm. group" suffered the most large γ-rays doses, began to show the turning-movements of embryo in egg membrane and their heart beats as normally as the control group did, but the next day (4 days after) they were all dead without hatching.

These observations were shown microphotographically in the Plate VII A1–5. As shown in Plate VIII, Figs. 5, 6, 7 and 8 in the "26 cm. group" we found the very peculiar form of deformities that the authors had never seen before, these were the curvatures of vertebrae or tail-portions and the hypertrophy of yolk-sac or the pericardia.

The extremes of them lay down at the bottom of the water and swam round and round at times, but soon after they were dead. Six days after irradiation the "26 cm. group" were annihilated.

In the "35 cm. group" we found the similar deformities of the young as the "26 cm. group". These deformities were shown in Plate IX, Fig. 6–9.

In the "85 cm. group", the degree of deformities was more slight than in the "26 cm. and 36 cm. group". The development and deformities of "85 cm. group" were shown in Plate X. In the "100 cm. group", the development was almost same as the control group, except a few minor deformities as shown in Plate XI.

Table 6. Relation between the quantity of projected γ-rays and the diameter of eggs (Exp. II). Measured immediately after irradiation, each figure in this table is the average of 10 tests.

r	Major	Minor
28,500	0.15 cm.	0.13 cm.
10,800	0.14	0.13
6,000	0.14	0.13
1,000	0.15	0.14
730	0.14	0.13
Control	0.15	0.14

Table 7. Relation between the quantity of projected γ-rays and the total length of the young (Exp. II). Measured after three and four days, each figure in this table is the average of 15 tests.

r	Irradiated after	
	3 days	4 days
28,500	— cm.	— cm.
10,800	—	—
6,000	0.44	0.48
1,000	0.48	0.52
730	0.46	0.52
Control	0.49	0.54

It is generally said in the ichthyic pathology that the curvature of vertebrae are due to the lack of an inorganic salt, and Vitamin A, except due to parasites.

The hypertrophies of the pericardia are not same as those of the hydropsy. The reason why the "16 cm. group" died before they hatched, may be the defect of the enzyme for hatching. But it is not evident because the experiment about this has not yet been carried out.

In the second experiment the infections were also clearly observed as in the first experiment namely with the increasing doses of radiation the mortality

of eggs and the abnormal hatch rate increased in number, and with the increasing doses of radiation the deformities were still more peculiar.

We measured the diameter of eggs immediately after irradiation in order to know the relation between the γ-rays irradiated doses and the development degree of eggs, and the results were shown in Table 6. And the relation between the irradiation doses and the total length of the young was observed the same as before, and the results were shown in Table 7. In these observations the delay of development was recognized, but it was not so distinct.

Summary

We attempted preliminary studies on the early development of the gold-fish (*Carassius auratus* L.) projected with Co^{60} 41 C. (γ-ray) radiation. In this experiment the eggs were projected with various quantities of γ-ray ranging from 730 r to 31,500 r.

The increase of quantity of the projected γ-ray the following observations were obtained during the early development of gold-fish.

(1) The decrease of normal hatch rate.
(2) The inclination of delay of development stage.
(3) Under more than 1,000 r. of γ-ray projection the eggs produced several appreciable deformities, such as the curvatures of vertebrae or tail, hypertrophy of yolk-sac or pericardium.

These results were given in the tables and shown in the plates microphotographically.

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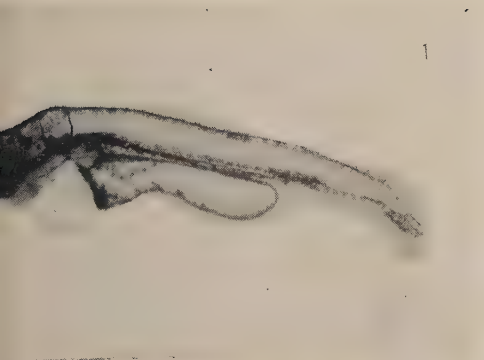
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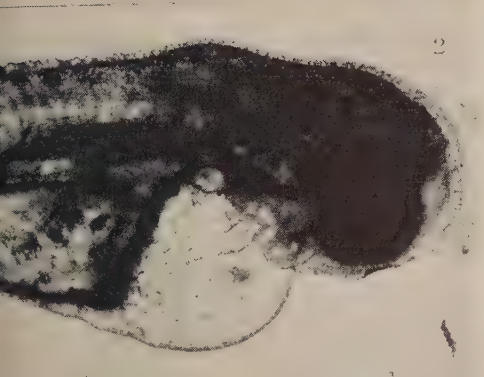
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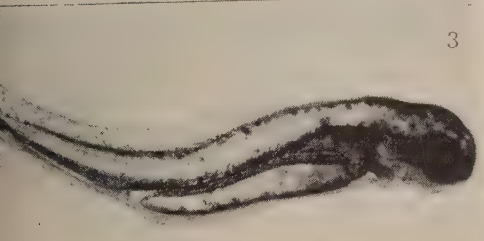
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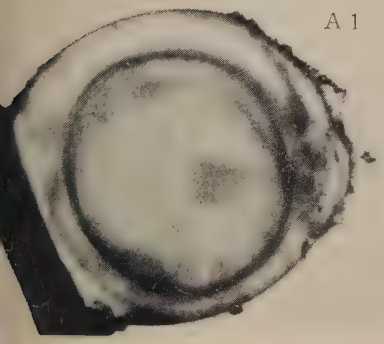
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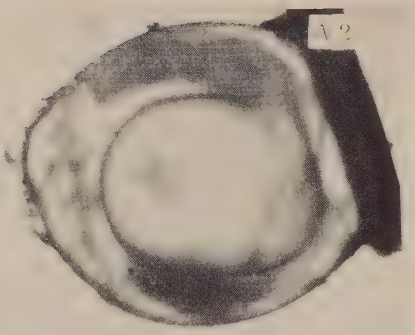
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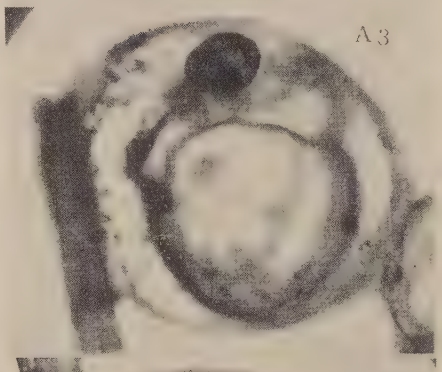
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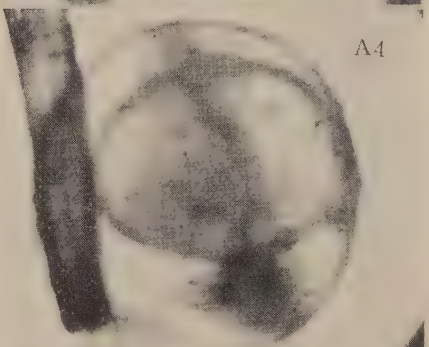
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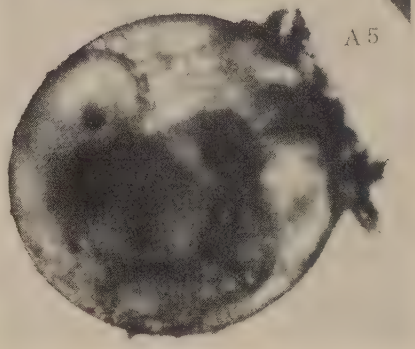

A 5

PLATE VII

Figs. 1–3. The major deformities of gold-fish five days after irradiation (Exp. I). Fig. 1. Irradiated with Co^{60} 41 C. distance from 35 cm. in 16 hours (irradiated dose Ca. 6,600 r). Fig. 2. Enlarged photo. of Fig. 1. Fig. 3. Irradiated with Co^{60} 41 C. distance from 85 cm. in 16 hours (irradiated dose Ca. 1,100 r). Fig. A1–5. The development of gold-fish (Exp. II). Irradiated with Co^{60} 41 C. distance from 16 cm. in 14.5 hours (irradiated dose Ca. 28,500 r). Fig. A1, immediatly after irradiation; A2, next day; A3, 2 days after; A4, 3 days: and A5, 4 days.

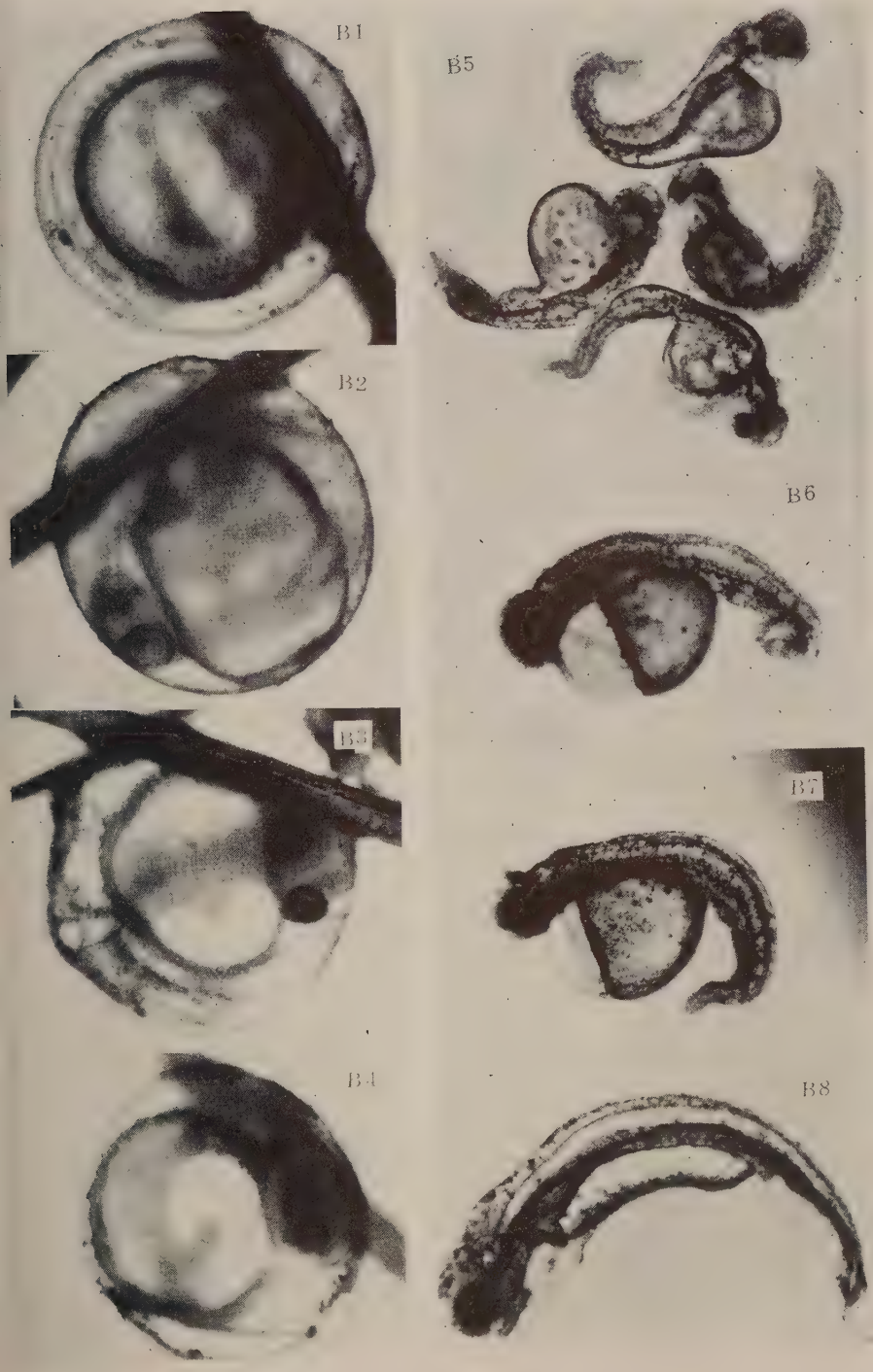
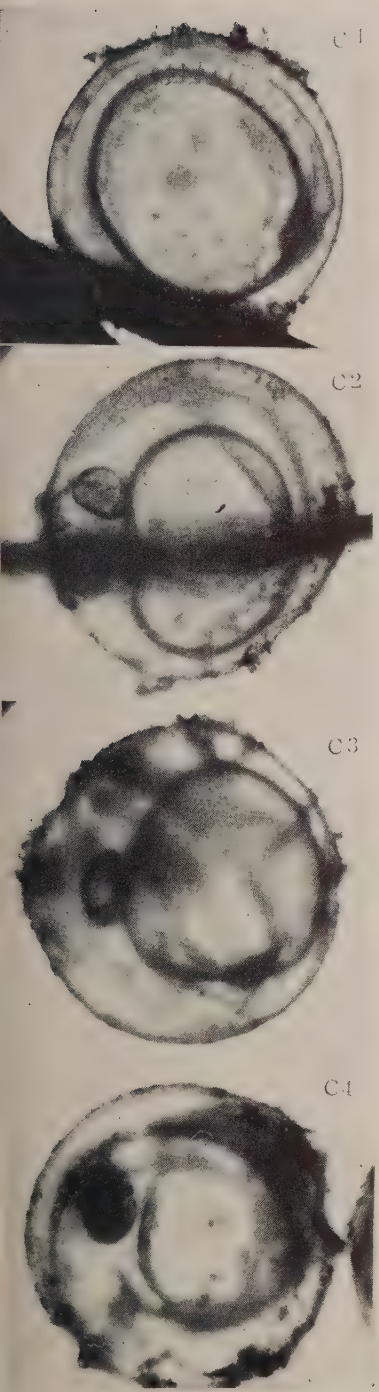

B1
B2
B3
B4
B5
B6
B7
B8

PLATE VIII

The development of gold-fish (Exp. II). Irradiated with Co^{60} 41 C. distance from 26 cm. in 14.5 hours (irradiated dose Ca. 10,800 r). Fig. B1, Immediately after irradiation; B2, next day; B3, 2 days after, B4, 3 days after; B5, 3 days after (young); B6, 7 and 8, 4 days after, B5, 6, 7 and 8 are the major deformities of the young, i.e. curvature of vertebrae, hypertrophy of pericardium and yolk-sac.


C1
C2
C3
C4

C5
C6
C7
C8
C9

PLATE IX

The development of gold-fish (Exp. II). Irradiated with Co^{60} 41 C. distance from 35 cm. in 14.5 hours (Irradiated dose Ca. 6,000 r). Fig. C1, Immediately after irradiation; C2, next day; C3, 2 days after; C4, 3 days after; C5, 3 days (immediately after hatching); C6, 7, 8 and 9 are the deformities of the young 4 days after irradiation.

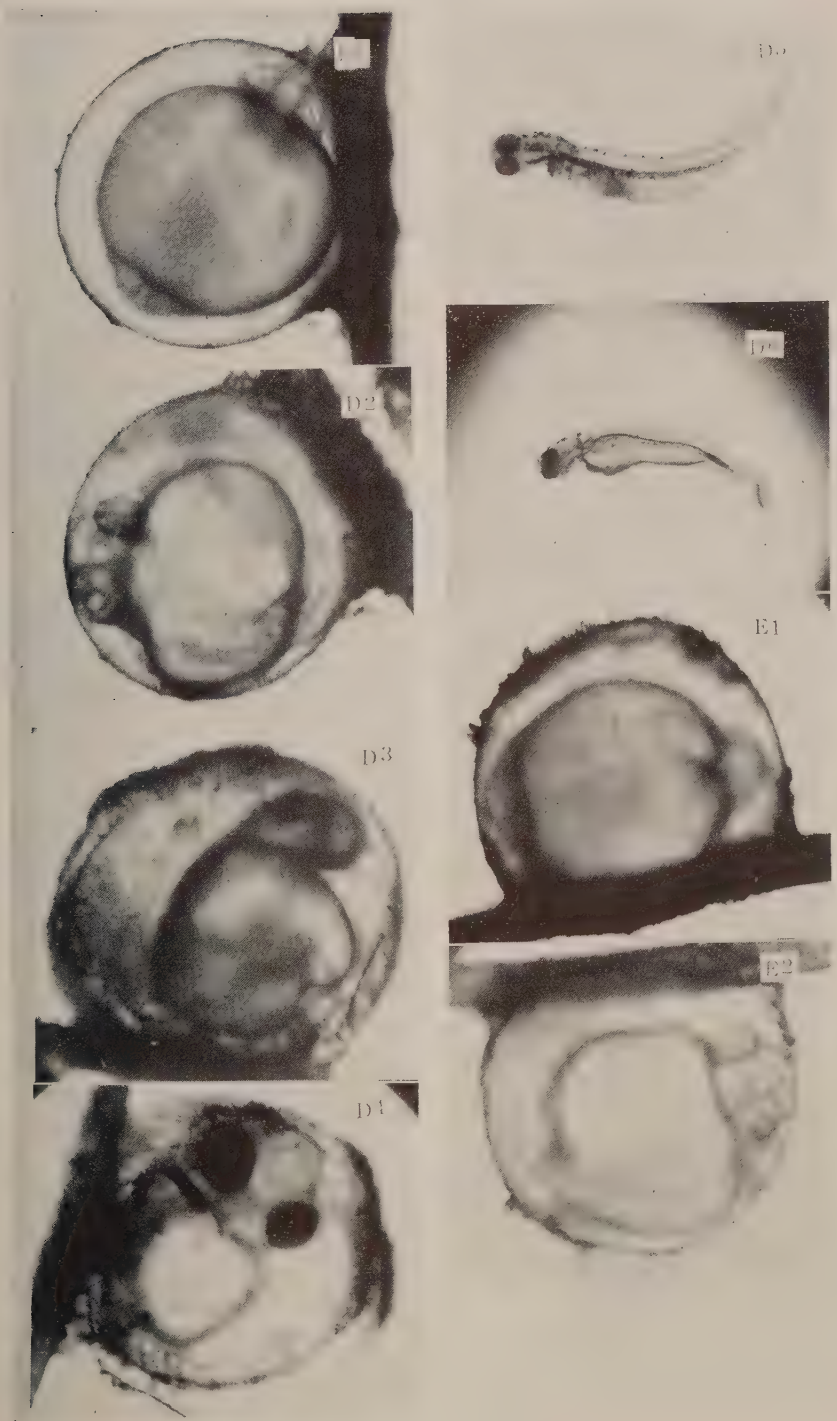
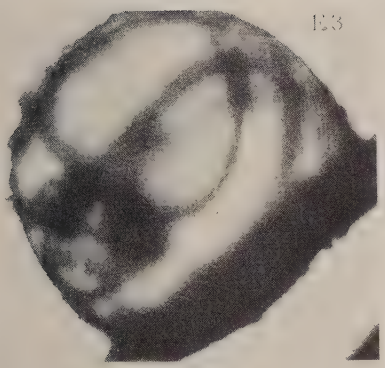
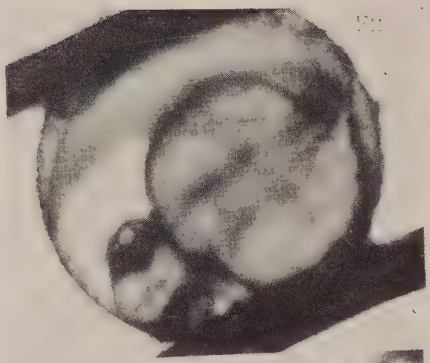
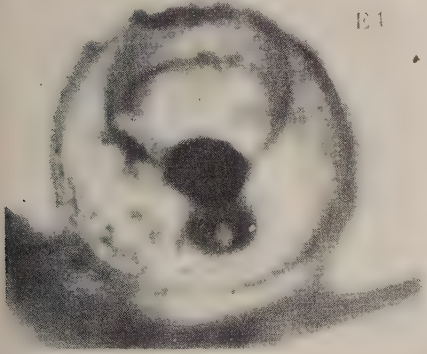

D1
D2
D3
D4
D5
E1
E2

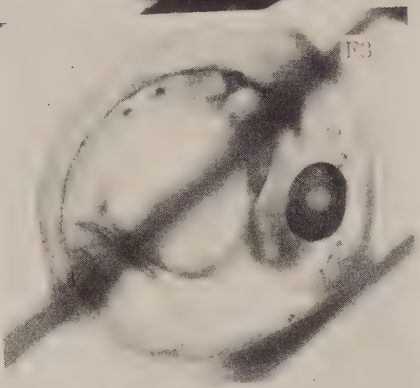
PLATE X

The development of gold-fish (Exp. II). Irradiated with Co^{60} 41 C. distance from 85 cm. in 14.5 hours (irradiated dose Ca. 1,000 r). Fig. D1, Immediately after irradiation; D2, next day; D3, 2 days after; D4, 3 days after; D5, and 6, 4 days after (the deformities of the young). Fig. E1–2, The development of gold-fish (Exp. II). Irradiated with Co^{60} 41 C. distance from 100 cm. in 14.5 hours (irradiated dose Ca. 730 r). Fig. E1, immediately after irradiation; E2, next day.

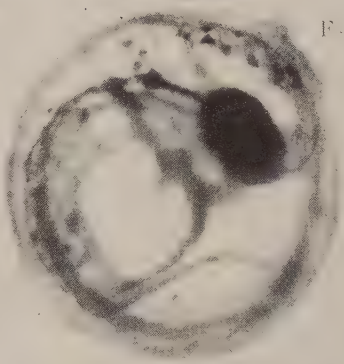

E3

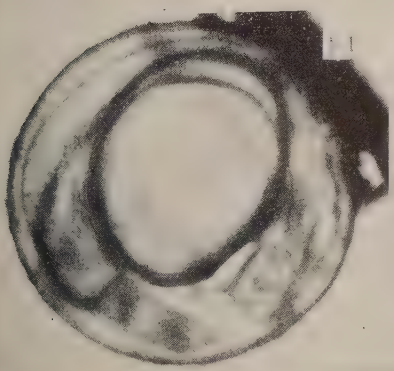

F2


E4


F3

E5


F4


E1

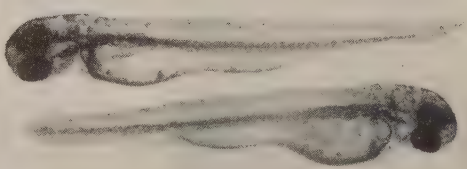

F5

PLATE XI

The development of gold-fish (Exp. II). Fig. E3–5 (continued from previous Pl. X). E3, 2 days after; and E4 and 5, 3 days after irradiation. Fig. F1–5. The development of gold-fish (Exp. II) control group. (no irradiation). Fig. F1, immediately after irradiation; F2, next day; F3, 2 days after; and F4 and 5, 4 days after.

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SPAWNING HABIT AND EARLY DEVELOPMENT OF A PUFFER, *FUGU (TORAFUGU) NIPHOBLES* (JORDAN ET SNYDER)

Yutaka Uno

(Received Jan. 10, 1955)

Introduction

The present account deals with spawning habit and early development of the *Fugu (Torafugu) niphobles* (Jordan et Snyder) (Abe, 1952) a puffer, commonly found in coastal waters of Japan. This fish has been of no practical use, since it is one of the most toxic among puffers. Highly specialized spawning habit of this fish renders its life history unique among those of other ones of the genus (Welsh and Breder, 1922), in which the fish is included, although it nearly resembles that of the grunion, Leuresthes tenuis, of which some detailed reports were set forth by Thompson (1919), Clark (1925), and Walker (1952).

As to the spawning habit of this puffer, few literatures are known to exist (Abe, 1950). The present writer was given an opportunity of observation on said subjects of this fish during May-July, 1952–'53, at Kominato Marine Biological Laboratory of the Tokyo University of Fisheries. The result arrived at by the present investigation may be given on.

The writer is indebted to Professor J. Hori of the University and Dr. T. Ino of the Tokaiku Fisheries Institute for their suggestion rendered during the course of this work. Special thanks are due to Professor Dr. I. Kubo of the University for his criticism and revision. Also the writer offers his gratitude to Messrs. M. Masuda and T. Furukawa for generous assistances in carrying out this investigation.

Material and method

The material used in this study was obtained at Kominato, Chiba Prefecture, by both seine and angling during the breeding season. For indication of body-length of the fish, the standard length was measured by using a pair of dividers. For weighing of body-weight a caliblated spring balance readable to the nearest gram was employed. For enumeration of mature ovarian eggs, the following method was employed, viz. $N=m\times W/w$, where N, total number of ripe ovarian eggs; n, number of eggs in unit weight of ovary; W, w, total and unit weights of ovary respectively. Diameter of ovarian ova was measured by means of an eye-piece micrometer after separation procedure of the Simpson's method (Simpson, 1951). Insemination was carried out at laboratory with ripe eggs taken out from the females caught at spawning bed and brought to the laboratory. Their development was observed under ordinary as well as holizontal microscopes.

In order to expose the hatching eggs to air in the moist, they were placed

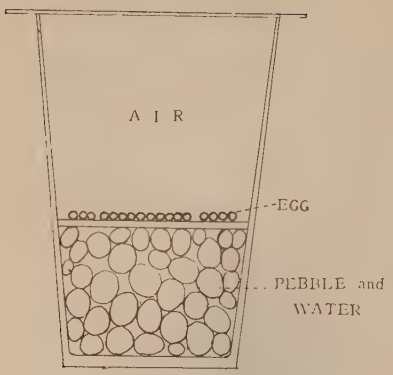

Fig. 1. Apparatus used for exposing eggs to air in the moist.

an apparatus devised by the author (Fig. 1). To know effect of temperature for the development in egg, an experiment was performed with TAUCHI's serial incubator.

Spawning habit

A) Spawning.

Spawning of the puffer was found to take place on the narrow pebbly beaches during high tides of the months from May to July. On the rising tide for 1–5 days after the new or full moon during such months given above, large schools of the fish come to the beaches. The Fig. 2 shows the relation between phases of the moon and the observations made on the spawning times during the period from May to July, 1953.

So far as the present investigation goes, the first small spawning run was observed on May 14 and the second during the period from two days before the full moon until five days after it. (May 21 to June 3). The third

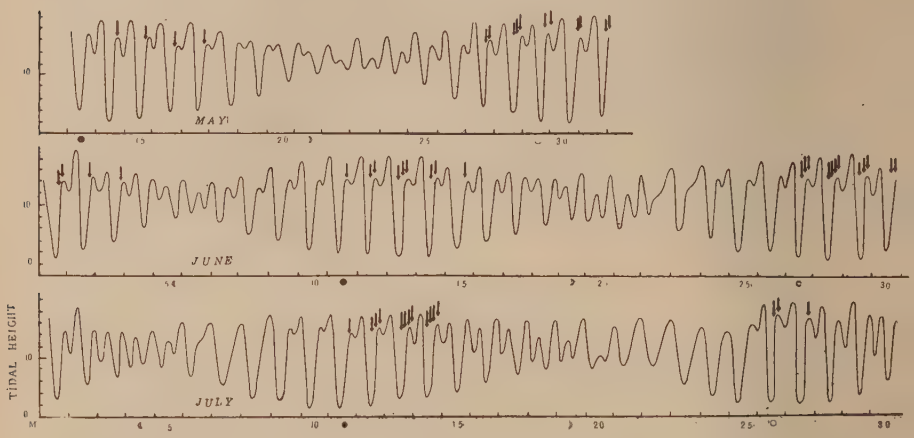

Fig. 2. Tidal levels reached each day at Kominato, Chiba Pref. The phases of the moon indicated. The observed spawning times of *Fugu* (*Torafugu*) *niphobles* indicated by arrows. Year 1953.

occurred on days from June 11 to June 15. The spawning runs were observed regularly at fortnightly intervals after the 3rd run. The spawning behavior is of very interest. Mature fishes come slowly in with the rising tide and gather near the beaches (Fig. 3, A). Then it seems that they are going back and forth at the edge of the water. Occasionally the puffers come to the

beach on the waves and are left on the pebbles as the water recedes, then sqirm and flop back stately into the wash of the next waves. The spawning usually starts about two hours before the full tide. At this time, 2–4 males make several attacks on the lateral surface of the trunk of a female.

Fig. 3. Spawning of *Fugu (Torafugu) niphobles* on the beaches.
A. The spawner coming to the beaches with rising tide.

Then the female escapes towards the beach, and the spawners frequently strand themselves on the beaches until another waves wash over them. (Fig. 3, D.) As soon as 2–4 males bite the lateral surface of the trunk of a female, the female lays eggs, the milt is extruded, and then fertilization of the eggs results. The actual process of egg-laying takes about 30 seconds. The peak of spawning is reached about a half hour after the beginning of the spawning. When the spawning activity was in full swing, the water of the spawning bed was found stained with the milt ejaculated by males. The spawning is repeated for about two hours before the sunset when the schools of fish scatter (Fig. 3, B-C). The eggs are wrinkled by the waves on the beach, suspended in the beach-comber, and carried underneath the pebbles by the outgoing tide. During the low tides, the eggs are left on the beach covered with pebbles or sands, thus staying out of water but remaining in moist sands for 5–6 hours during the night and 8–9 hours in the daytime in the course of a day (Fig. 3, E).

Fig. 3. B–C. The fishes spawning in a cloud of spray on the beaches.

Fig. 3. D, A male biting a female

Fig. 3. E, Numerous eggs beneath the pebbles.

Fig. 3. F, Wringing out mature ova.

B. Egg-eating habit.

For the purpose of evidencing this habit, 752 specimens caught carefully by a seine on the spawning bed were dissected and their alimentarly canals, in which many eggs are often contained, were examined. They were frequently found to be full of eggs. Investigation revealed that 23.1% of 138 females and 32.4% of 612 males are found with stomach containing eggs laid by themselves. It is definitely established by this fact that the fishes which come to spawning bed eat eggs after spawning is completed. Further, the body-lengths of egg-

Table 2. Results of T- and F-tests of body-length of the fishes conducted in connection with egg-eating habit. N, number of specimens; $\bar{x}$, average value of body-length in cm.; V, variance; F_0, variance ratio from F-table (Tokei Kagaku Kenkyu Kai, 1952; F, variance ratio calculated; df, degree of freedom; t_0, value from t-table; t, value of student's t calculated.

Sex		Factor N	Factor $\bar{x}$	Factor V	F-test	T-test df	T-test t	T-test $t_0(\alpha=0.01)$
♀	E_0	32	10.94	3.09	$F=1.06<F_0^{200}_{32}(0.01)=1.64$	136	2.95	2.62
	E_1	106	12.02	3.31				
♂	E_0	198	10.32	1.52	$F=1.25<F_0^{500}_{200}(0.05)=1.33$	609	6.30	2.62
	E_1	413	11.09	1.96				

eating fishes (E_0) are shorter than those of non-eating ones (E_1). In the former the body-length measure 10.94 cm in females and 10.02 cm in males on average, while in the latter those are 12.02 cm and 11.09 cm on average in females and males respectively. This differences are significant at 5% of risk by analysis of variance (Masuyama, 1947). Values obtained are shown in the Table 2.

Remarkably enough, the male were nearly four times as abundant as the females in the catch by seine. Those males and females are 10.86 cm and 11.76 cm in body-length on average respectively. But this difference is denied within one percent of risk (Table 3 and the Fig. 4).

Table 3. Result of F- and T-test of the body-length of the fishes conducted in connection with spawning.

Sex	Factor			F-test	T-test		
	N	$\bar{x}$	V		df	t	$t_0(\alpha=0.01)$
♀	138	11.76	3.57	$F=1.82<F_{100}^{400}(0.01)=1.28$	750	5.3	2.58
♂	613	10.86	1.96				

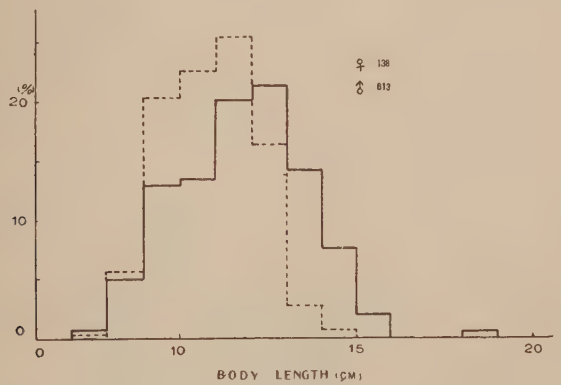

Fig. 4. Frequency polygon showing the number of fishes taken from spawning bed. Solid: female, broken: male.

C. Frequency of spawning and the number of mature ova.

In order to investigate the frequency of spawning of an individual fish in a spawning season, tagging experiments were carried out during May 14–17. Tag was put on the tail fin of the fish caught from the spawning bed. The result taken reveals that the males spend milt every day during this period and continue to do so on the following three series of high tides after the first period, and that the females lay eggs once on their first visit to spawning bed, and do not contain mature eggs at least in the next three spawning periods, although they visit there. Measurement of immature ova taken from three females on May 14, the beginning of the spawning season, has disclosed that the frequency distribution of the diameter is trimodal, having peaks at 520–233 μ, 192-150 μ and

150–27 μ. Those peaks are shown in the Fig. 5 as marked by a, b and c. The ova belonging to those 3 groups undoubtedly existed in the gonad when the first mature ova had been spawned, but it is yet to be determined whether immature ova reach maturity in the spawning season.

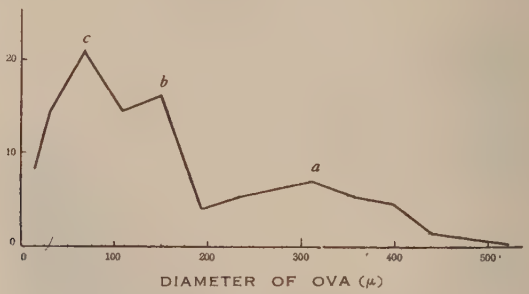

Fig. 5. Frequency distribution of overian egg diameter in percentage.

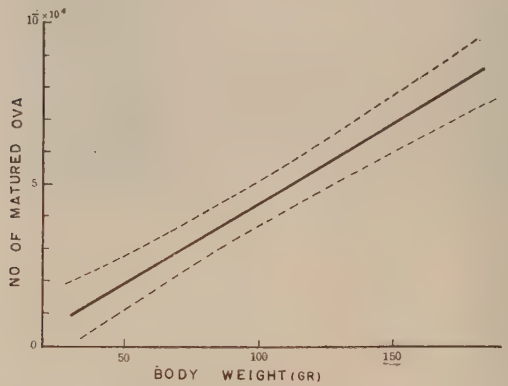

Fig. 6. Relationship between body-weight and number of mature ova. Solid line, calculated curve; dotted lines, 95% confident interval.

Table 4. Relationship between body-weight and number of mature ova. S.D., standard deviation.

Body-weight (gr)		No. of mature ova		No. of sample
Mean	S.D.	Mean	S.D.	
25.1	—	6798	—	1
42.6	7.5	15843	1281	3
54.7	2.5	24831	6988	7
85.2	6.4	38255	8231	5
123.2	18.0	74144	12351	6
165.1	18.5	85308	3656	3
187.4	6.6	92320	4814	2

The number of mature eggs counted in 27 specimens, 25.0–194.0 gr in body-weight, ranged from 6,789 to 92,320, as shown in the Table 4 and the Fig. 5. The relation between the number (N) and body-weight (W) may be expressed by the formula, $N=a-b$, where a and b represent constants. Computing the approximate values (Deming 1943), the following relation be obtained, $N=492W-4778$. And 95% confident belt will be calculated as follow: $\pm 1727(39.5-0.434W+0.0022W^2)^{1/2}$. In this connection it should be mentioned herewith that the most diminutive female examined measured 25.0 gr in weight and 9.3 cm in body-length.

D. Consideration.

As stated already, the spawning habit of this fish undoubtedly is controlled by tidal cycles. The heights of tides vary according to the phase of the moon, the highest tides occurring when the moon is full or new. Also the two high tides of each days vary in height along the Pacific coast of Japan. During the spawning season the lower high tides were in the daytime. The puffer spawned only on those lower high tides, and after the tide has started to rise as shown in the Fig. 7, while the grunion (Walker, 1952) always spawns on a receding

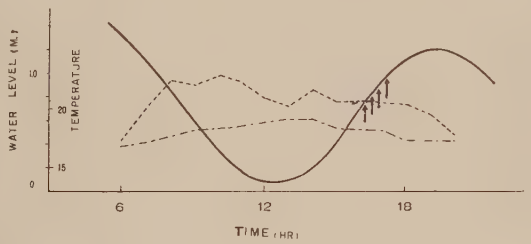

Fig. 7. Tidal curve at spawning time (May 16, 1953). Tidal curve:—; water temperature:—·—; Air temperature:·····; Spawning time:←.

tide during the night. From the observations made on the spawning actions of the fish, it seems that they commence spawning under the stimuli elicited by both biting the lateral surface of the trunk of female and rubbing their ventral side against pebbles in the surfs. The habit, however, most closely approaches those of the grunion, *Leuresthes tenuis* of California (Thompson, 1919), *Habbsiella sardina* of the Gulf of California (Walker, op. cit.) and *Galaxias attenuatus* of Australia (Hefford, 1931a, 1931b.) in that the spawning times are adjusted to moon phases, although the *Leuresthes* spawns only during the night, *Hubbsiella* on both high tides and *Gallaxias* does not spawn on the beaches.

Clark (1925) by a series of measurements of the ova taken from grunion has ascertained that after an individual fish starts spawning it continues to spawn periodically on each series of high tides throughout the spawning season. In the case of the present species, however, females come to the spawning bed do not spawn at least on three series of high tides after the first spawning. Whether the ovarian ova which are contained among the gonad after spawning mature and are spawned during the course of spawning season, will be a subject of future research.

The fact that the smaller fishes show a tendency to eat eggs will be of

some interest and importance for the fundamental study of Puffer's toxin in view of the Tani's report (1940) that the toxicity is ordinarly strong in the spawning season.

As described above, the eggs are suspended in the surf, buffeted by the waves and exposed to the element for 5–8 hours in the course of a day. That the eggs can survive under these circumstances will be because their capsule has become hard enough to withstand the variations in the environment and the embryos can develope well in the air provided that the humidity is high as will be seen later. These properties will be taken to indicate that the eggs are endowed with the ability to adapt themselves remarkably to the surroundings as is the case with grunion. It is the author's opinion that this adaptability has been brought about the fish's peculiar spawning habits.

Early development of the fish and its period of hatching.

A. Development.

Eggs are spherical in shape, light brownish in color and $976\pm0.87\,\mu$ in diameter. It is possible to draw a line between blastderm and yolk sphere in which numerous colorless oil-globules lie one upon the other. A pattern of wavy network is observed on the surface of the egg chorion. It becomes more defined with growth of egg. The normal course of the development of the eggs which are reared under an average temperature of 21.7°C, is as follows: (Fig. 8).

Time elapsed after insemination.		Details of the developmental processes.
1 hr.	30 min.	The formation of the blastdisc begins.
2	00	2-cell stage.
2	40	4-cell stage.
3	30	8-cell stage.
4	20	16-cell stage.
7	15	Morula stage.
13	00	Blastrula stage.
16	00	Gastrula stage, growth of the epiboly reaches about one fourth of the egg surface.
18	00	The germ ring reaches near the equatorial position.
20	00	Growth of the epiboly reaches about four fifths of the egg surface.
30	00	The blastpore closed and the optic vesicles are well defined.
39	00	The embryo has about eight somites and the Kupffer's vesicle is making its first appearance. The black pigment shows the first signs.
44	00	The embryo has 10–12 somites and the pupiles are seen in both eyes. The auditory vesicles make their appearance.
50	00	The embryo has about 15 somites. The Kupffer's vesicle decreases in size.
65	00	The embryo has about 20 somites and Kupffer's vesicle

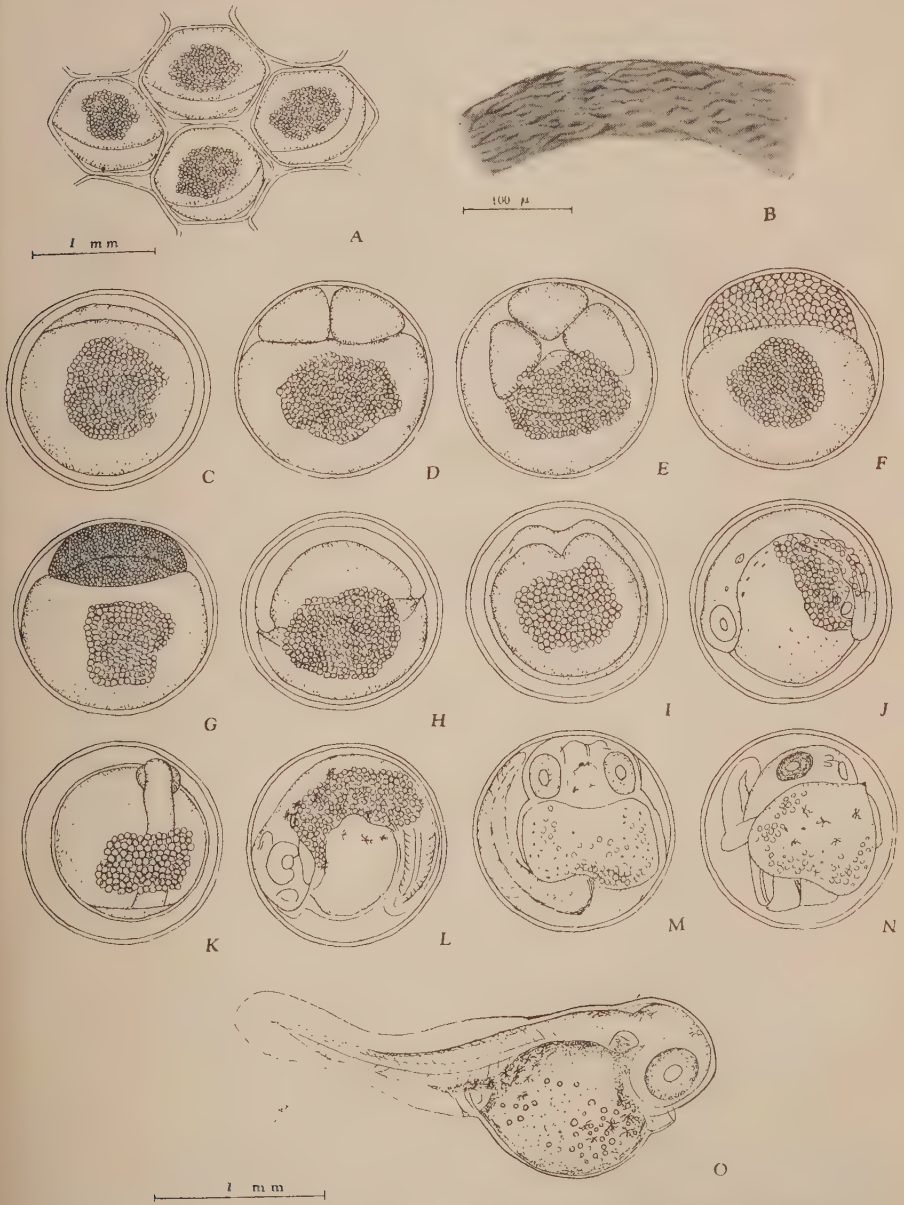

Fig. 8. Early development of *Fugu (Torafugu) niphobles*. A, unfertilized egg; B, wavy network on the surface of the egg chorion; C, egg just fertilized; D, 2-cell stage, 2 hours; E, 4-cell stage 2 hours 40 minutes; F, blastrula stage, 13 hours; G, gastrula stage, 16 hours; H, embryo with germ ring having reached near an equatorial position, 18 hours; I, embryo after 30 hours; J, embryo having optic vesicles, 39 hours; K, embryo with auditory vesicles and Kupffer's vesicle, 44 hours; L, embryo after about 60 hours; M, embryo after about 96 hours; N, embryo after about 135 hours; O, larva just hatched, 2.06 mm in total length.

		has disappeared. The tail has started to grow free of the yolk sac.
90	00	The tail of the embryo encircles two thirds of the egg membrane. The black pigment has appeared in the eye and the yellow pigments are observed on the surface of the head and yolk-sac of embryo. The heart pulsation is well observed.
110	00	The embryo has reached a stage where it has so grown hat its encircling tail almost reaches the eyes. Spasmodic movements of the body can be seen.
135	00	Pectral fin well grown. The lens of eyes are observed. The movement become more vigorous than the previous stage.
164	00	The larva hatched in about seven days after insemination. The lava is 2060±36 μ in total-length.

B. Effects of temperature on the development.

With a view to determining the effects of temperature on the developing eggs, the inseminated eggs were reared under 10 different variations of temperatures ranging from 10.65 to 29.47°C. The Table 6 gives the result of this experiment. The temperature suitable for hatching ranged from 21.37 to 27.9°C.

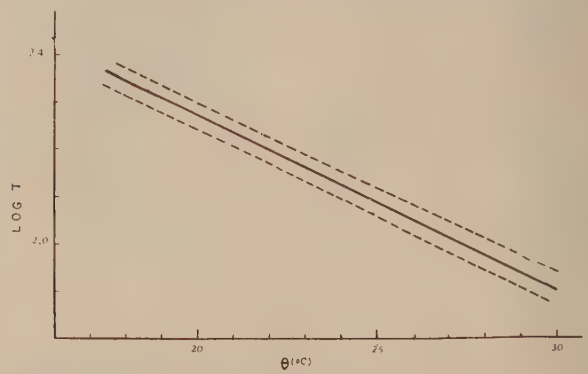

Fig. 9. Relationship between period of hatching and temperature. Solid line, calculated curve; dotted lines, 95% confident interval.

The optimum temperature was about 26.5°C. Under the suitable temperatures the period of hatching was 90–165 hours and the hatching rate of fry ranged 50% when the temperature was higher than 29.47°C or lower than 19.37°C, and the eggs perished when the temperature was lower than 14.6°C. The relation between the temperature (θ) and the time in hour (T) required for hatching may be expressed in favor of Higurashi-Tauti's formular as follows (Higurashi and Tauti, 1925).

$$Te^{a\theta}=c \quad i.e. \quad \log T=\log c-0.434a\theta$$

where θ, T and a represent the temperature, period of hatching and the constant, respectively. Calculating approximate values of a, c and Q_{10} from the Table 6, the following equations will be obtained (Deming, 1943).

$$\log T = 3.0061 - 0.03568\,\theta,$$

$$a = 0.084; \qquad Q_{10} = 2.3\ .$$

Table 6. The effects of the temperature upon hatching time and hatching rate of eggs under 100% moisture.

Temperature (°C)		Time of hatching (hr.)		Hatching rate (%)
Mean	S.D.	Mean	S.D.	
29.47	0.61	91	1.5	47.2
27.90	0.52	99	4.0	89.2
26.53	0.72	109	6.5	96.8
24.77	0.56	117	5.0	90.9
23.55	0.20	137	8.0	88.3
21.37	0.55	165	7.5	84.3
19.37	0.55	230	9.5	47.8
16.96	0.67	254	3.0	15.6
14.06	0.79	—	—	0
10.56	0.89	—	—	0

The 95% confident belt will be calculated as follows: $\pm 8.252(1.9\times10^{-5}-8.6\times10^{-7}+1.98\,\theta^2)^{1/2}$. The value of Q_{10} and a are close to those of sea-parrot, *Calotomus japonicus* (Seno et al. 1925).

The exposed eggs reared under different varieties of temperature were then put back into the water (water temperature 24.5°C), and their hatching state was observed. The result is shown in the Table 7. The eggs kept under 24.77±0.5 (group I) hatched within 4 hours; the eggs put under 21.37±0.55 (group II), 8 hours; the ones placed under 19.37±0.55°C (group III), 16 hours.

Table 7. Hatching state of exposed egg.

Time elapsed after being put back in the water (hr.)	Hatching state (%)			
	group I	group II	group III	group IV
1	58.3	44.0	10.2	63.9
2	—	14.6	—	—
3	27.4	—	41.6	25.0
4	14.3	21.1	—	—
5	—	—	—	11.1
8	—	20.3	—	—
16	—	—	24.5	—
No. of the eggs examined	321	373	366	231
Hatching rate (%)	90.9	83.4	47.8	15.6

The hatching rate of them were 90.9, 84.3 and 47.8%, respectively. On the other hand, the egg reared under 16.96±0.67°C (group IV) hatched within 5 hours, but the hatching rate was only 15.6%. It will be concluded that the hatching time is lengthen and the hatching rate falls off under the influence of lower temperatures.

C. Consideration.

As mentioned above, the eggs exposed to the air in the moist can develop and its suitable temperatures range widely from 21.37 to 28.0°C, on the other hand, water temperature during May and June of the spawning area range from 18.8 to 20.9°C, which are considerably lower than the optimum set forth above. It might not be amiss to presume, however, that the temperature under which the eggs develop in natural environment would be almost similar to the optimum temperature because they are exposed to the warm air for 8–9 hours in the course of a day. As is well known, the eggs of grunion can withstand for changes of the surroundings (Thompson, op. cit.). Puffer's eggs will, in like manner, be able to adapt themselves to the change of the circumstances such as great change in salinity, temperature and moisture. This will be a subject of further study from the standpoint of physiology.

Summary

An investigation of spawning habits and the early development of *Fugu* (*Torafugu*) *niphobles* has been undertaken during the spawning season of this fish in 1952 and 1953. The consequences of the present study are summarized as given below.

1. The spawning season extends from early May to late July in the vicinity of Kominato. The puffer ordinarily spawns only on 1–5 days following each full or new moon, at rising tide.

2. In the spawning bed, smaller ones of this fish eats eggs laid after spawning.

3. The males caught in spawning beds are about four times as abundant as the females, and smaller than the other ones. The former 10.87 cm and the latter 11.76 cm on average of body length. But this difference is denied within one per cent of risk.

4. The males spend milt every day during one or three series of high tides is succession. Meanwhile the females appear to discharge whole eggs in one spawning period, although they come to the beaches on each series of high tides after spawning. No ripe eggs are found to exist in the females spawned once.

5. The relationship between body-weight (W) and number of mature ova (N) may be represented by the following formula, with 95% confident belt:

$$N=492W-4778\pm1727(39.5-0.434W+0.0022W^2)^{1/2}\ .$$

In Early spawning season, there exists three groups of immature ova in which diameter runs 520—233 μ, 192—150 μ and 150—27 μ.

6. The mature ova are settling and semi-adohesive. It is possible to draw

a line between blastderm and yolk sphere before fertilization. The inseminated eggs are 976.0±0.87 μ in diameter and furnished with wavy marks on their outer surfaces. The marks become more defined with growth of egg. Cleavage is discoidal as shown in the Fig. 8.

7. The eggs exposed to air in moist can develop, and the range of temperature suitable for hatching varies from 21.37 to 27.9°C. The optimum temperature is about 26.5°C. The relationship between the temperature and the period of hatching may be expressed by the following formula given by Higurashi-Tauti, $Tc^{0.0840}=1014$. The temperature coefficient for embryonic development of the fish is found to be $Q_{10}=2.3$.

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SYSTEMATIC STUDIES ON THE JAPANESE MACRUROUS DECAPOD CRUSTACEA. 5. A NEW PALINURID, *NUPALIRUS JAPONICUS*, GEN. ET SP. NOV.

Itsuo Kubo

(Received Jan. 15, 1955)

A remarkable palinurid lobster was caught by an obscure fisherman in October, 1953 at a depth of about 200 m about 8 miles off Shimokawaguchi, Kôchi Prefecture while the fisherman was collecting coral. Dry specimen of the lobster was sent to the present writer for identification by Professor Jûjirô Ishikawa of Kôchi Prefecture Women's College.

This palinurid is found very similar to *Justitia longimana* (H. Milne-Edwards) in general feature, but differs greatly in many respects. The discrepancies appear for the present author to be so considerable as to establish new generic and specific categories for reception of this remarkable crustacean. Since *Nupalirus japonicus* gen. et sp. nov. is erected herein, and descriptions and some discussions may be given later on.

Acknowledgements should be mentioned herewith for wholehearted assistance of Messrs. Kazunori Takagi and Jin Hattori of our laboratory for preparation of photographs and others. My hearty thanks are also due to Professor Dr. Arata Terao of the Miyazaki University, by whom valuable suggestions were made for designation of the new generic name and to the Ministry of Education as the expence of the present investigation is partly defrayed by the Grant in Aid for Fundamental Scientific Research of the Ministry.

Nupalirus gen. nov.

Carapace furnished with squamiform sculptures; a median, a pair of supra- and infra-orbital spines present on frontal border, and any spines absent on the border between median and supra-orbital spines. Supra-orbital spines dorsally serrate. Abdominal segments each with many (about 4-7) transverse furrows. Telson longer than broad. Antennular flagellum short. Mandibular palp 3-segmented. First pereiopods not extremely robust and elongate, not subchelate; dactylus not strongly curved backwards. Outer uropods greatly elongated. Stridulating organs present.

Genotype: *Nupalirus japonicus* sp. nov.

The present new genus closely allies to the genus *Justitia* established by Holthuis (1946). But it is easily assortable from *Justitia* as it shows principal discrepancies in the following respects:—(a) having no intermediate spines on the anterior border of carapace between the median and supra-orbital spines; (b) first pereiopod not extremely stout and elongate, and not subchelate, dactylus not strongly recurved posteriorly; and (c) outer uropods remarkably elongated.

Nupalirus japonicus sp. nov.
(New Japanese name: Ryoma-ebi)

Shell hard; that of carapace, sixth abdominal segment, anterior part of telson, and antennal peduncle furnished with squamous sculptures; and that of pleons from first to fifth having 4-7 transverse grooves on tergum and characteristic sculptures on pleura of these pleons as shown Pl. XII, Upper. Each sculpture of carapace and antennal peduncle frinded with short bristles on anterior and antero-lateral margines, but on the contrary, that of sixth abdominal segment and telson provided with similar setae on posterior and postero-lateral edges.

Antennular tergum narrow and naked, without spines and bristles, but carries well developed lateral upheavals which form a part of the stridulating organs. Carapace subpentagonal in cross-section, with round dorsal surface and flat lateral one; provided with a median, a pair of supra-orbital, and a pair of infra-orbital spines on frontal margin; median spine smallest among those spines, sharply pointed at tip, reaches to posterior edge of antennular tergum; supra-orbital spines largest of the spines lying on frontal rim of carapace, extending well beyond distal end of second antennal segment, lateraly compressed, more or less curved downwards in distal half, armed with 2 short stout spines on upper edge; infra-orbital ones stout, rather short, with acute apex; 3 median spines and 5 pairs of spines present behind the median spine of frontal edge of carapace; 2 and 1 spines lying afterwards each supra- and infra-orbital spines respectively (Pl. XII, Lower). Cirvical groove well defined, has no spines on its posterior edge. Epistom afforded with a small pointed median spine on anterior border and a protuberance with round apex on each antero-lateral angle. Last thoracic sternum carrying 2 small spines on frontal border and 6 large acute spines on posterior edge, these 6 spines directed backwards. Pleonic pleura ending into a large, robust, acuminate spine (Pl. XII, Upper). Sternum of first pleon armed with 4 large, acute spines on posterior border, those of the pleons from second to fifth bearing 2 ones similar to those of the first pleon, that of sixth pleon provided 2 small spines on anterior rim and 3 ones on posterior border. Telson subrectangular in outline, with rounded distal margine, about one and half times as long as wide at base (Pl. XIII, Lower).

Antennule a little shorter than carapace. Antennal peduncle very stout; stridulating process of first peduncular segment well developed; distal outer angle of last peduncular segment protruded into a large depressed spine obliquely truncate on apex. Mandibular palp consists of 3 joints, 2.9 mm in each basal and last joint, and 6.5 mm in middle joint. All maxillipeds furnished with well developed exopodite reaching base of antenna; exopodite has a multiarticulate flagellum on tip. Third maxilliped 7-jointed, 3-4 small pointed spines present along inner edge of each basis and merus, and 8 ones on the same edge of ischium. All pereiopods afforded with an acute spine on distal outer angle of merus. First pereiopod attains distal end of intermediate segment of antennal peduncle; merus carries a spine on proximal outer border and on posterior inner edge about 15 ones which become gradually larger in size distally; carpus with a spine on distal inferior angle and several spines on posterior inner

border; propodus has about a dozen spines on posterior inner and outer edges, and a rather large spine near distal end of posterior surface. Second pereiopod somewhat surpassing distal end of intermediate joint of antennal peduncle. Third one reaches about distal extremity of second joint of antennal peduncle. Fourth one attaines near anterior end of basal segment of antennal peduncle. Fifth legs shorter than fourth ones; coxa has on inner border a slender styliform prolongation which is directed inwards, nearly reaches to median line of sternum on which fifth legs are set, and bears male genital aperture at distal end.

Pleopod leaf-shaped. Inner uropods slightly longer than telson. Outer ones remarkably larger than telson, about 1.7 times as long as telson.

Dimensions of some bodily parts are given below. Bodylength measured from anterior border to distal end of telson 237.6 mm; carapecial length 83.6 mm; length of telson 41 mm; length of antennular peduncle 61 mm, and that of first, second, and third joints 34, 11, 16 mm respectively; lengths of amburatory legs: 103 mm in first leg, 111.5 mm in second leg, 110. 5 mm in third leg, 100 mm in fourth one, 89 mm in fifth one; those of last 4 segments of first leg 41, 19, 22, 17 mm, and the same of third one 41, 16, 23, 17 mm respectively; length of inner uropods 43 mm, that of outer one 70 mm.

Holotype: male, deposited in the biological museum of Kôchi Prefecture Women's University.

Type locality: about 8 miles off Shimokawaguchi (Shimizu city), Kôchi Pref., Japan.

The present new species has, in general feature, much resemblance to *Justitia longimana* (H. Milne-Edwards) known from Antilles (H. Milne-Edwards, Gruvel), Cuva (von Martens, 1872), Santa Cruz (Bouvier, 1925) Mauritius (Miers, 1882), Oahu, Hawaii (Edmondson, 1951). But differences are found to exist on many traits besides those enumerated already as generic character of the new genus *Nupalirus*. Of which principal ones are as follows:—Spines of posterior border of cervical groove are absent (present in *J. longimana*). Sculpture pattern of the sixth abdominal segment is somewhat more intricate than that of *J. longimana*. The spine of the distal outer corner of the last peduncular segment of antenna is much larger than that of *J. longimana*. All other pereiopods, except the first one, are considerably thicker than those of *J. longimana*.

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PLATE XII.

Nupalirus japonicus, gen. et sp. nov., male, carapace-length 83.6 mm.
Upper, lateral aspect; Lower, upper aspect of cephalothorax.

PLATE XIII.

Nupalirus japonicus, gen. et sp. nov., 83.6 mm in carapacial length.
Upper, dorsal aspect; Lower, ventral aspect.

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SOME PROBLEMS ON THE FISHING ABILITY OF PURSE SEINE FLEETS FOR SARDINE FISHERY AS RELATED TO THE TONNAGE OF THE BOATS, THE SIZE OF PURSE SEINE AND MATERIALS

Makoto Inoue

(Received Oct. 10, 1954)

In this paper the author wishes to describe the results of observations on the purse Seine fleets for sardine fishery at Chiba Prefecture as related to the quantity of catch per day, the number of daily pursing and the amount of catch during each pursing. Also he wishes to discuss on the fishing ability of the purse Seine fleets in relation with the tonnage of the boats, the size of the nets and the materials for it.

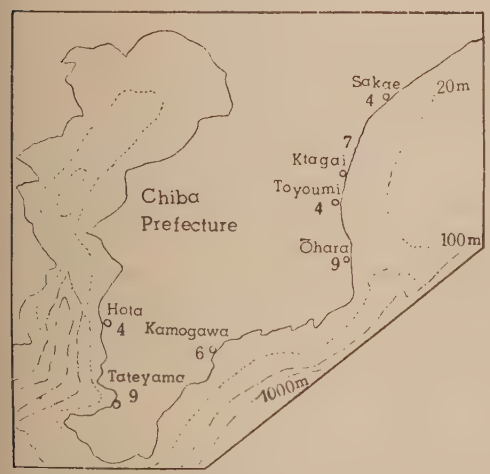

Fig. 1. Position of the stations where investigations were carried out along the coasts of Chiba Prefecture. The numerals indicate the number of fleets and the dotted lines the bathymetrical contour.

The stations where investigations were carried out are illustrated in Fig. 1.

Employing the calculation method of Dr. M. Tauti (1950), the author obtained the following results.

The relative values for the quantity of catch, the number of pursing, the amount of catch per pursing for each day are shown in Tables 1–7.

In cases where the number of pursing for each day was insignificant as shown in Tables 5–7, the author took the average number of pursing in a catch instead of the relative values.

From the results of observations the following discussions may be concluded.

1) The differences in fishing ability for each boat among the Katagai fleets are shown in Table 2. From this Table it is clear that the respective relative values are, 0.84 to 1.07 for the amount of catch, 0.91 to 1.14 for the number of pursing, and 0.93 to 1.11 for the amount of catch per pursing. It is difficult to explain the variation in the relative values of the mentioned items, because there is no remarkable difference in the tonnage of the purse boats of the Katagai fleets, and also no noteworthy difference exists in the size of the

net used, the number of the crew, nor in the kind of fish finder.

2) With regard to the effect of the kind of materials of which the nets are manufactured on the fishing ability, it is noticed that the purse seine fleets which used cotton nets showed a slightly smaller value than that of Cremona (vinylon), as indicated in Table 3. Such a tendency is recognized in the season extending from May to September, while in other seasons there is little difference.

No remarkable difference in the value of the amount of catch among nets manufactured by Cremona, Kanebian (vinylon) and those of cotton was recognized, as shown in Tables 4 and 6. From Table 5 it is clear that Amillon (nylon)nets showed the same value as ones consisting of cotton.

Owing to that nets made of Kanebian, Cremona or Amillon need not be dried for preservation, their daily use is an advantage over those which require drying for their preservation. However, such advantage is contrary to the results, because there was found no remarkable difference in the number of pursing per day for each boat which used nets regardless of being manufactured with Cremona, Kanebian, Amillon or cotton.

3) In Chiba Prefecture, the purse seine fleets may be classified into two types according to the tonnage of the boats, namely, 4–7 ton class and about 20 or more tons class. In the former class, a crew of 20–40 men utilize from one to two fish finders and purse a net of 82–100 Ken long with 40-60 strips. However, in the latter class, a crew of 50–60 men use only two to three fish finders and purse a net of 115–135 Ken long with 58–85 strips.

Accordingly, it is reasonable to discuss on the fishing effect of the tonnage of the boats, the size of the nets separately as shown in Tables 1, 3 and 6. Thus, although there is no significant difference between the two classes in the number of pursing each day, it is remarkable that the catch per pursing of the 20 or more tons class is 1.4–2:3 times greater than that of the 4-7 tons class.

From the available data there can be found no remarkable difference in the fishing ability of purse seine due to the material of which the nets are manufactured.

In Chiba Prefecture, purse boats of about 20 or more tons catches 1.4–2.3 times more than boats of the 4-7 tons class, and the number of the crew and size of the nets are nearly two times greater in the former than in the latter.

Finally, the author wishes to express his hearty thanks to Professor Dr. M. Tauti for his kind advice and encouragement during this study and to Professor Dr. H. Niino for kindly reading the manuscript.

Table 1. Sakae.

Elements of pursing seine fleets and its relative value on fishing at Sakae.

Fleet		A	B	C	D
Tonnage of seine boat		4.3 t	19.6	19.8	19.5
Horse power of engine		10 HP	40	50	40
Nets		Cotton	〃	〃	〃
Length of cork line (one half)		94 K*1)	120	122	120
Size of mesh and number of strips*2)		cm 1.3, 40	1.3, 58	1.3, 75	1.3, 75
Number of crew		30	50	60	50
Number of fish finder		1	2	2	2
Season		Nov., 1953～Feb., 1954			
Relative value	Catch	0.53	1.36	1.08	1.03
	Number of pursing	1.07	1.13	0.97	0.83
	Catch per pursing	0.58	1.16	1.09	1.17

*1) 1 ken = 1.5 m.

*2) A strip has one hundred meshes.

Table 2. Katagai.

Fleet		A	B	C	D	E	F	G
Tonnage of seine boat		19.7 t	19.9	19.7	19.6	19.2	18.0	19.5
Horse power of engine		50 HP	45	50	55	50	40	50
Materials of Nets		Cotton	〃	〃	〃	〃	〃	〃
Length of cork line (one half)		135 K	130	132	128	128	133	132
Size of mesh and number of strips		cm 1.3 80	1.3, 78	1.3, 80	1.3, 75	1.3, 75	1.3, 78	1.3. 80
Number of crew		54	54	56	56	58	60	56
Number of fish finder		2	2	3	3	3	3	2
Season		Oct., 1952～Sept., 1953						
Relative value	Catch	1.07	1.07	1.07	1.07	0.84	0.93	0.95
	Number of pursing	1.01	1.14	1.06	1.01	0.91	0.96	0.91
	Catch per pursing	1.04	0.93	1.00	0.99	0.93	1.00	1.11

Table 3. Toyoumi.

Fleet		A	B	C	D
Tonnage of seine boat		6.8 t	19.05	19.0	19.8
Horse power of engine		12 HP	50	40	65
Nets		Cotton	〃	Cremona	Cotton Amillan
Length of cork line (One half)		78 K	136	140	138
Size of mesh and number of strips		cm 1.3, 65	1.3, 75	1.3, 80	1.2, 80
Number of crew		30	58	60	60
Number of fish finder		2	3	3	3
Season		Jan.～Mar., 1953 & Oct.～Dec., 1953			
Relative value	Catch	0.53	1.17	1.20	1.10
	Number of pursing	1.00	1.00	1.03	0.97
	Catch per pursing	0.50	1.08	1.19	1.23
Season		May～Sep., 1953			
Relative value	Catch	0.84	0.90	1.37	1.07
	Number of pursing	1.08	0.89	1.01	1.02
	Catch per pursing	0.77	0.92	1.43	0.88

Table 4. Ōhara.

Fleet	A	B	C	D	E	F	G	H	I
Tonnage of seine boat	25.5 t	25.4	25.4	19.9	26.5	23.5	15.5	25.5	27.1
Horse power of engine	70 HP	70	70	70	80	65	60	90	80
Nets	Kanebian	Cotton	〃	〃	〃	〃	〃	〃	〃
Length of cork line (One half)	135 K	125	130	128	120	128	130	135	130
Size of mesh and number of strips	cm 1.3, 80	1.3, 73	1.3, 75	1.3, 75	1.3, 75	1.3, 76	1.3, 65	1.3, 80	1.3, 76
Number of crew	60	60	58	56	58	56	30	60	58
Number of fish finder	3	3	2	2	3	2	1	3	3
Season	Nov., 1952～Apr., 1953								
Relative value: Catch	1.25	0.86	0.97	0.80	1.12	0.82	0.58	1.21	1.17
Relative value: Number of pursing	1.09	0.94	1.05	0.94	1.00	0.99	0.80	1.07	1.04
Relative value: Number per pursing Catch	1.15	0.91	0.99	0.77	1.08	0.92	0.68	1.18	1.13

Table 5. Kamogawa.

Fleet	A	B	C	D	E	F
Tonnage of seine boat	28.0 t	25.5	23.0	6.3	6.5	6.3
Horse power of engine	70 HP	50	50	15	12	12
Nets	Amillan	Cotton Amillan	Amillan Kanebian	Cotton	〃	〃
Length of cork line (one half)	124 K	124	120	86	82	85
Size of mean and number of strips	cm 0.8, 80	0.9, 75	0.8, 79	1.2, 60	1.2, 58	1.2, 60
Number of crew	60	58	60	40	35	35
Number of the fish finder	3	3	3	2	2	1
Season	May～Oct., 1953					
Relative value of catch	1.37	0.97	1.39	0.82	0.81	0.64
Season	Mar.～Nov., 1953					
Relative value of catch	1.09	0.81	1.10			
Average number of pursing	2.7	2.6	2.9			

Table 6. Tateyama.

Fleet	A	B	C	D	E	F	G	H	I
Tonnage of seine boat	19.0 t	19.3	19.3	19.9	17.8	15.5	16.0	19.0	4.1
Horse power of engine	40 HP	50	80	60	50	40	40	50	12
Nets	Cremona	〃	〃	Kanebian	〃	Cotton	〃	〃	〃
Length of cork line (one half)	117 K	120	117	120	117	117	115	117	100
Size of mesh and number of strips	cm 1.3, 80	1.2, 80	1.3, 80	1.2, 85	1.3, 80	1.1, 80	1.2, 80	1.2, 75	1.3, 60
Number of crew	55	55	60	60	55	56	55	56	20
Number of fish finder	1	1	1	1	1	1	1	1	1
Season	May～July, 1953								
Relative value of catch	0.94	0.96	1.26	1.28	0.88	0.93	0.77	0.92	
Average number of pursing	3.5		4.0	4.2	3.4	3.5	3.3		
Season	Nov.～Dec., 1953								
Relative value of catch	0.95	0.93	1.06	1.36	0.90	1.10	0.99	1.04	0.61
Average number of pursing	2.9			3.2	3.0		3.0	2.9	2.3

Table 7. Hota.

Fleet	A	B	C	D
Tonnage of seine boat	18.5 t	18.9	19.6	19.0
Horse power of engine	60 HP	50	40	50
Nets	Cotton	〃	Cotton Kanebian	Cotton
Length of cork line (one half)	130 K	117	120	117
Size of mesh and number of strips	cm 1.3, 90	1.3, 75	1.3, 80	1.3, 75
Number of crew	60	55	56	56
Number of fish finder	1	1	1	1
Season	May～July, 1953			
Relative value of catch	1.19	1.00	0.96	0.85

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MODEL EXPERIMENTS ON FIXED NETS FOR AMBER-FISH, *SERIORA*, USED IN THE COAST OF MIE PREFECTURE.

Katsuji Honda and collaborators*

(Received Jan. 10, 1955)

To perform, in laboratory, a model net investigation of a fixed net for Amber-fish, *Seriora*, we made a preliminary investigation on the said nets at Mie Prefecture during Jan. 7 to 16 in 1953. The nets examined during that time are as follows:

Nishiki-ichigô	Otoshi-ami[1)]	(Net I)
Shimakatsu	Otoshi-ami[2)]	(Net II)
Togashirajima	Otoshi-ami[3)]	(Net III)
Kuki-ichigô	Otoshi-ami[4)]	(Net IV)

Model net investigations were this time carried out to see what deformations these nets may suffer and what condition of tidal current may be critical for maintaining a good balance between the actions of their floats and sand bags, model nets corresponding to the actual ones being tested under various speed vectors of artificial tidal current. In this investigation was also measured the total horizontal resistance exerted by each whole net.

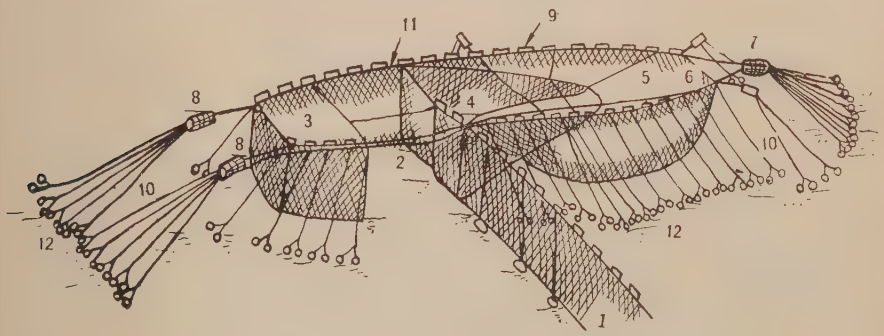


Fig. 1. The term of "Otoshi-ami."

1. Kaki-ami (Lead net)
2. Haguchi (Entrance of net)
3. Undoba
4. Nobori-ami (Funnel net)
5. Hako-ami (Bag net)
6. Uodori (Landing net)
7. Dai-aba (Float)
8. Yabiki-aba (Float)
9. Gawa-aba (Float)
10. Dohyo-zuna (Sand bag line)
11. Gawa-zuna (Cork line)
12. Dohyo (Sand bag)

The essential structure of Otoshi-ami is referred to Figure 1. For grasping detailed feature of the net employed in the present work Tables 1–4 and

* A. Koike, M. Suzuki, N. Hirayama, M. Nonoda, M. Nakamura, K. Nakamura, A. Yoshikawa, S. Tamai, K. Hara, H. Kato, Y. Sugawara, U. Iidaka, J. Nishimura, M. Matsuura, and M. Ishigaki.

Figure 2 will be available. In constructing the model nets the conditions having been required by the TUTI's[5] law concerning fishing nets, *i.e.*, so-called "the law of similarity," were fulfilled as given below between a model net and the corresponding actual one. (Of course the angle between every two adjacent arms were made identical for both the cases, the model and the actual. It should be noticed also that, while the netting cord of the actual net was

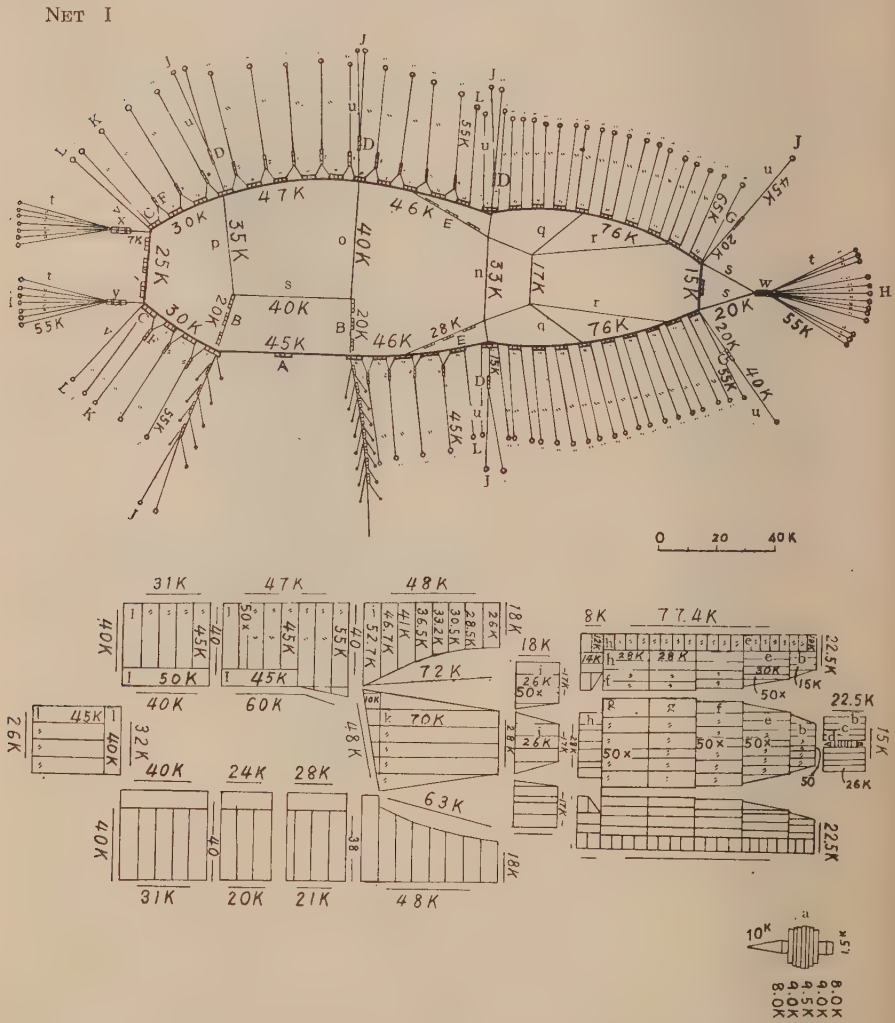
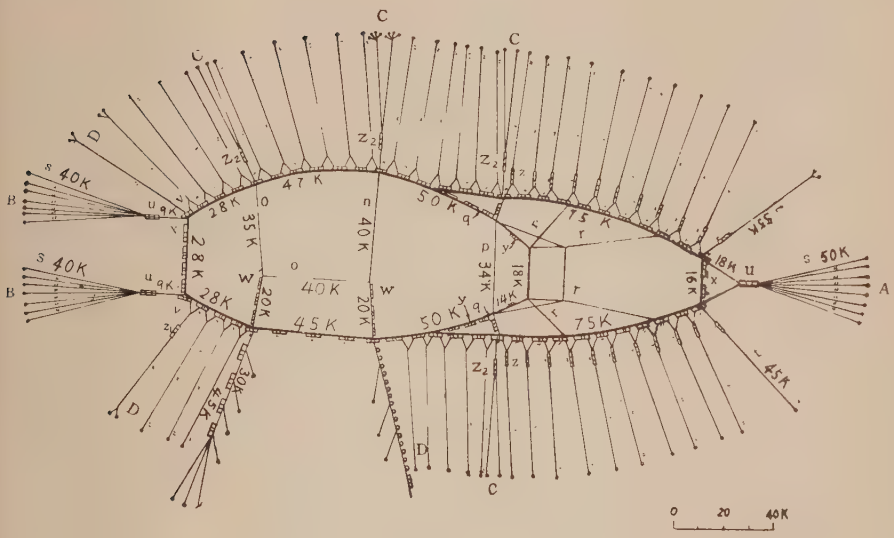


Fig. 2. Structure of the "Otoshi-ami (Net I~IV). The symbols are used throughout Tables in this report. The Arabian numerals with K (=Ken≒1.5m) show the length of rope or net, and ones with × the number of meshes of a strip net, while other nets without × are of 100 meshes.

NET II


C
C
C
D
D
D
A
B
B
40K
40K
28K
28K
28K
47K
50K
75K
40K
45K
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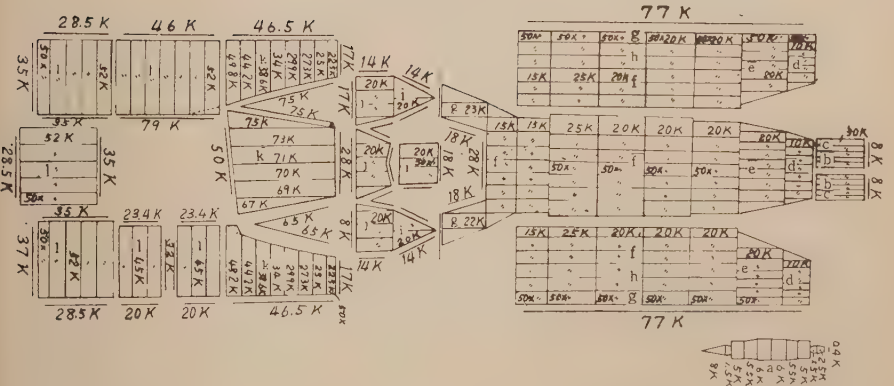
16K
14K
55K
50K
45K
30K
0 20 40K


77K
28.5K
46K
46.5K
35K
79K
52K
28.5K
35K
23.4K
23.4K
37K
28.5K
20K
20K
46.5K
14K
14K
17K
20K
75K
73K
71K
70K
69K
67K
65K
50K
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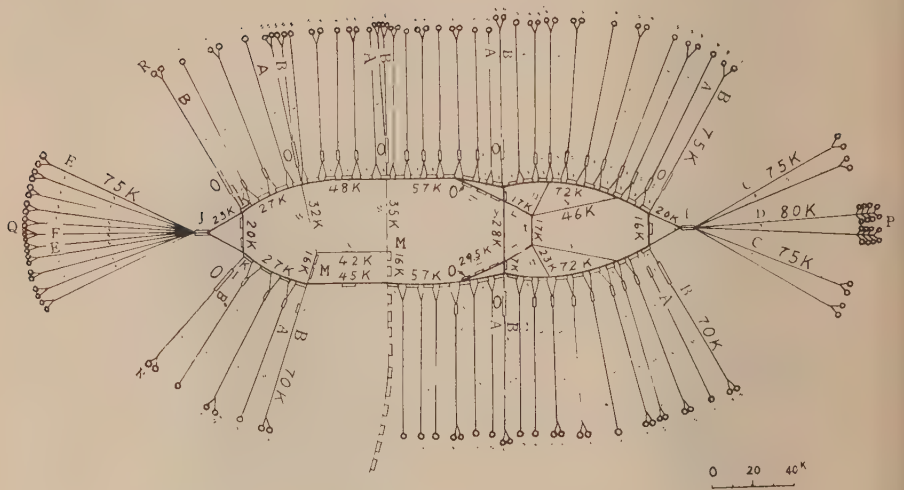
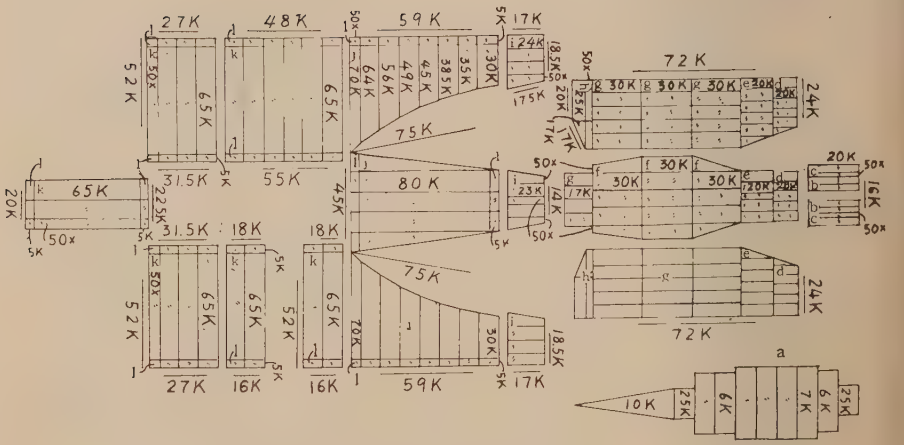
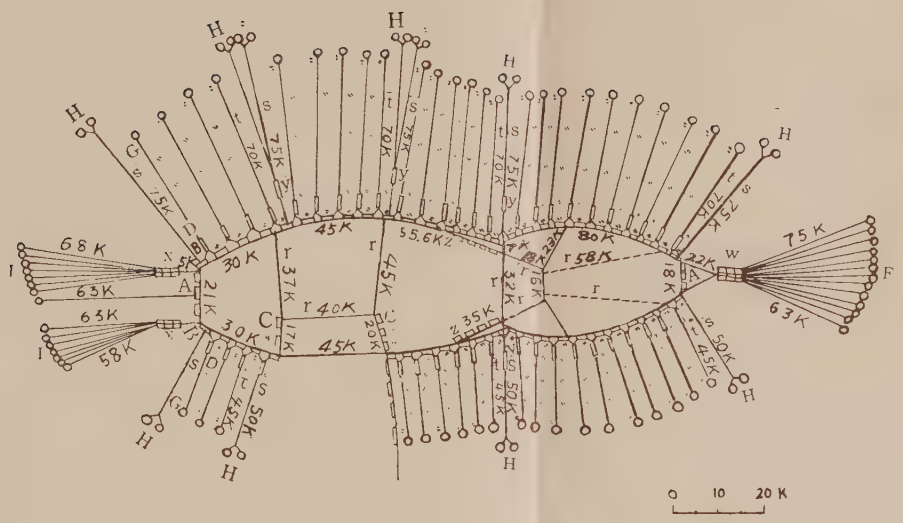
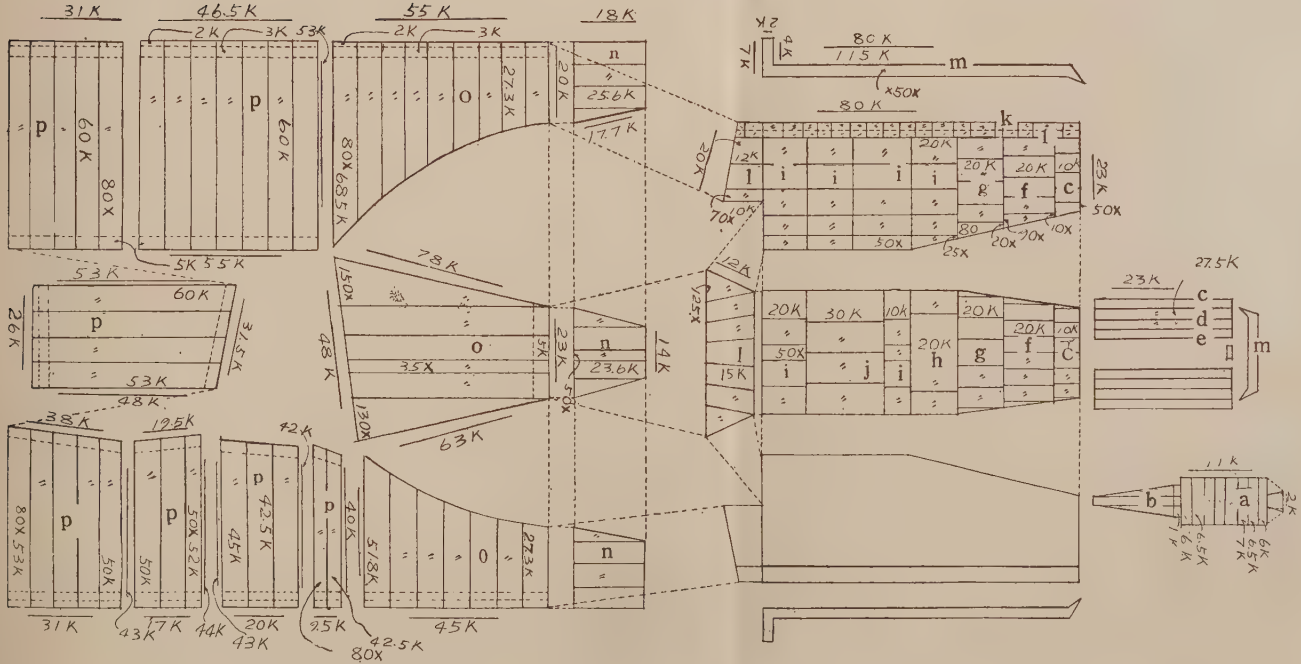
18K
8K
77K
50K
25K
20K
15K
8.5K
30K
0 20 40K

NET III






H
68 K
63 K
63 K
58 K
75 K
63 K
45 K
55.6 K
80 K
58 K
45 K
0 10 20 K


31 K
46.5 K
55 K
18 K
80 K
115 K
m
80 K
k
l
i
g
f
c
50 K
53 K
60 K
80 K
26 K
37.5 K
48 K
78 K
150 K
48 K
35 K
23 K
14 K
63 K
38 K
125 K
42 K
80 K
45 K
27.5 K
23 K
c
d
e
m
b
a
11 K
2 K
6.5 K
6 K
7 K

of manila hemp and the actual ropes were of manila-hemp or steel wire, those of the model consisted of cords either of silk or cotton.

1) Since a reducing scale $\lambda' : \lambda'' = 1:500$ was adopted, λ' and λ'' expressing the dimensions respectively of a model net and of the corresponding actual one, each model was made so as to be of dimensions overall in this ratio.

2) Denoting by D' and D'' the diameters, and L' and L'' the mesh lengths respectively of a model and the actual net, the following equations hold between the two sizes of net.

$L'/L'' = D'/D'' = .12$ in the case of Net I, III, and IV.

$,, = ,, = .11$ in the case of Net II.

The values in this connection are listed in Table 1.

3) Indicating the current velocities by v' and v'', and specific gravities of the material constituting netting cord by ρ' and ρ'' respectively of a model and the actual net, and furthermore the specific gravity of fresh water in the experimental tank by ρ_w' and that of sea water on the actual fishing ground by ρ_w'', the relation between v' and v'' can be given by the following equations, where ρ' is 1.25 (for silk) and ρ'' 1.41 (for Manila hemp).

$v'/v'' = \sqrt{D'/D'' \cdot (\rho' - \rho_w')/(\rho'' - \rho_w'')} = \sqrt{.12 \cdot {}^{1.25-1.00}/_{1.41-1.025}} \fallingdotseq .27$ in the case of Net I, III, and IV.

$,, \quad ,, \quad ,, = \sqrt{.11 \cdot {}^{1.25-1.00}/_{1.41-1.025}} \fallingdotseq .26$ in the case of Net II.

4) Let D_1' and D_1'' denote the diameters, and ρ_1' and ρ_1'' the specific gravities respectively of the thread in the model and of the rope in the actual net (ρ_1'' being 7.83 for wire rope). Then we get the relation

$D_1'/D_1'' = \sqrt{\lambda'/\lambda'' \cdot (v'/v'')^2 \cdot (\rho_1' - \rho_w')/(\rho_1' - \rho_w'')} = \sqrt{{}^1/_{500} \cdot (.27)^2 \cdot {}^{1.41-1.00}/_{1.25-1.025}}$
$\fallingdotseq 1.5 \times 10^{-2}$
in the case of Net I, III, and IV.

$,, \quad ,, \quad ,, = \sqrt{{}^1/_{500} \cdot (.27)^2 \cdot {}^{1.41-1.00}/_{1.39-1.025}}$
$\fallingdotseq 1.2 \times 10^{-2}$
in the case of Net I, III, and IV.

$,, \quad ,, \quad ,, = \sqrt{{}^1/_{500} \cdot (.27)^2 \cdot {}^{7.83-1.00}/_{1.39-1.025}}$
$\fallingdotseq 5.1 \times 10^{-2}$
in the case of Net I, III, and IV.

$,, \quad ,, \quad ,, = \sqrt{{}^1/_{500} \cdot (.26)^2 \cdot {}^{1.41-1.00}/_{1.25-1.025}}$
$\fallingdotseq 1.1 \times 10^{-2}$
in the case of Net II.

$,, \quad ,, \quad ,, = \sqrt{{}^1/_{500} \cdot (.26)^2 \cdot {}^{7.83-1.00}/_{1.25-1.025}}$
$\fallingdotseq 6.1 \times 10^{-2}$
in the case of Net II.

The values calculated are recorded in Table 2.

5) Denote the buoyancy due to floats in the model and such in the actual net by F' and F'', respectively. Then $F'/F''=\rho_w'/\rho_w''\cdot(\lambda'/\lambda'')^2\cdot(v'/v'')^2$ $={}^{1.00}/_{1.025}\cdot({}^{1}/_{500})^2\cdot(.27)^2\fallingdotseq.29\times10^{-6}$ in the case of Net I, III, and VI.

$F'/F''=\rho_w'/\rho_w''\cdot(\lambda'/\lambda'')^2\cdot(v'/v'')^2={}^{1.00}/_{1.025}\cdot({}^{1}/_{500})^2\cdot(.26)^2\fallingdotseq.27\times10^{-6}$ in the case of Net II.

Table 3 contains the figures in this concern.

6) Show the weight of the lead used in the model and that of sand bags in the actual net respectively by w' and w'' which are reduced in water to be w_1' and w_1''. Then, expressing the fixing forces of these two kinds of sinkers distributively by r' and r'', there will hold the correlation, because the values such as $w':w_1'$ and $w'':w_1''$, are known as given in the right portions of the equations:

$$w'/w''=(\lambda'/\lambda'')^2\cdot(\rho_w'/\rho_w'')\cdot(v'/v'')^2\cdot(w_1''/w_1')\cdot(r''/r')=({}^{1}/_{500})^2\cdot({}^{1.00}/_{1.025})\cdot(.27)^2\cdot({}^{.65}/_{.912})\cdot({}^{.63}/_{.44})\fallingdotseq.30\times10^{-6}$$

for Net I, III, and IV.

,, ,, ,, $=({}^{1}/_{500})^2\cdot({}^{1.00}/_{1.025})\cdot(.26)^2\cdot({}^{.65}/_{.912})\cdot({}^{.63}/_{.44})\fallingdotseq.28\times10^{-6}$

for Net II.

In the case of a funnel net, since there will work no fixing force due to sinker, we get

$$w'/w''=(\lambda'/\lambda'')^2\cdot(v'/v'')^2\cdot(\rho_w'/\rho_w'')\cdot(w_1''/w_1'')=({}^{1}/_{500})^2\cdot(.27)^2\cdot({}^{1.00}/_{1.025})\cdot({}^{.65}/_{.712})\fallingdotseq.29\times10^{-6}$$

for Net I, III, and IV.

,, ,, ,, $=({}^{1}/_{500})^2\cdot(.26)^2\cdot({}^{1.00}/_{1.025})\cdot({}^{.65}/_{.712})\fallingdotseq.28\times10^{-6}$

for Net II.

The evaluations lead to the figures shown in Table 4.

In our laboratory runs, a net model without attachment of lead net (Kaki-ami) was first so set in an experiment-tank with a glass-window that it assumes its regular configuration in a water current sufficiently mild and of normal direction (*cf.* footnote on page 201). Water in the tank was strictly adjusted of its depth at the entrance of net after the TAUTI's law of similarity taking into accout the actual scale (*cf.* the representations of the actual state on fishing ground given in Figure 3).

Then the water was let to flow uniformly passing through the model net, while the rate of flow was also controlled along the requisite by the said law. Now changing the current velocity, *i. e.*, in magnitude as well as in direction, critical conditions were observed for various anomalies apprehended to occur, *e.g.*, distortion of net configuration, dragging of sinkers, etc. The states of model nets during experiment as photographed from a side as well as downward are illustrated in Figure 4.

From the above experimental data it may be certain that, in the actual

field too where delicate observations are in general next to impossible, net configuration begins to be deformed early while tidal current is still quite weak (1/6 knot per hour). With increasing rate of the current, floats being fixed at the upper edge of net tend readily to be pulled down into the water. When the current becomes very strong, whole the float line goes submerged so deeply

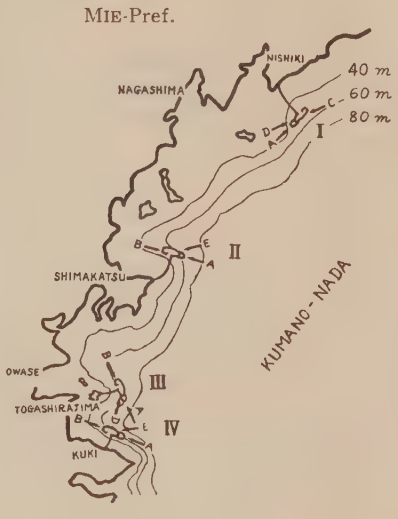


Fig. 3. Showing map of the fishing grounds where the "Otoshi-ami" are set.
I: Nishiki-ichigo, II: Shimakatsu,
III: Togashirajima, IV: Kuki-ichigo
→ : Current direction.

that the net as a whole flattens, being forced towards the sea bottom. It is evident in such a condition that the net has entirely got out of its function. As soon as the speed of current rises up to a certain critical value, the action of sinkers becomes no longer effectual owing to an immense tension laid upon the fixed net; and some of the sinkers, especially those which have been disposed upper the net against tidal stream, are more or less readily dragged away, resulting after all in an away-drift of the entire net.

Values of current velocity in critical condition have been calculated on the described observation in laboratory; they are summarized in Table 5. From the table it may in general be remarked that when tidal current flows deviating in direction appreciably from the normal condition as assigned *A* or *B*** the net becomes deformed more or less readily of its configuration, while the sand bags joined to the portions upper the net against tidal stream are particularly apt to be drifted off, making it difficult to keep the net in its regular conformation.

Data of the tidal velocity observed from 1945 to 1947[6] are listed for reference in Table 6.

** The normal direction of current, *A* or *B*, has been so defined that it agrees practically with the direction perpendicular to the surface of landing-net (Uodori) which constitutes a part of our actual net.

Horizontal stresses on model nets (R's) at various current velocities (V's) were measured side by side by means of a device illustrated in Figure 5. In this apparatus a silk string is laid stretched in parallel with the water line, being led from Dai-aba (F) which works as a float, through a pully (P) to a spring balance (S) so that the measurement may be attained after the motion of a needle on the balance pointing to an adjacent scale (L). The readings so obtained for models of Nets III and IV are further computed in terms for the actual nets and shown in Figure 6. On the basis of these data a correlation is found between R's and V's as expressed in an equation:

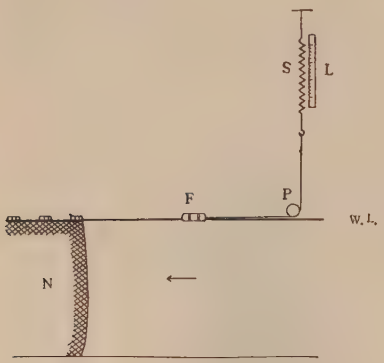


Fig. 5. Shematic diagram of the apparatus used for measuring tension. S: Spring balance, L: Scale, P: Pully, F: Float (Dai-aba), N: Net, ← : Current.

$$R = kV^n$$

where k and n are constant numbers. They can be determined from Figure 6, leading to the values given in Table 7.

As seen in Table 7, values of k and n are different from one type of net to the next.

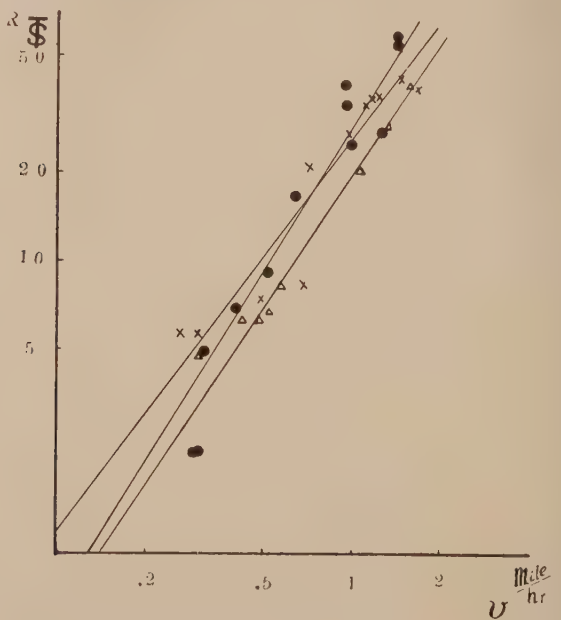
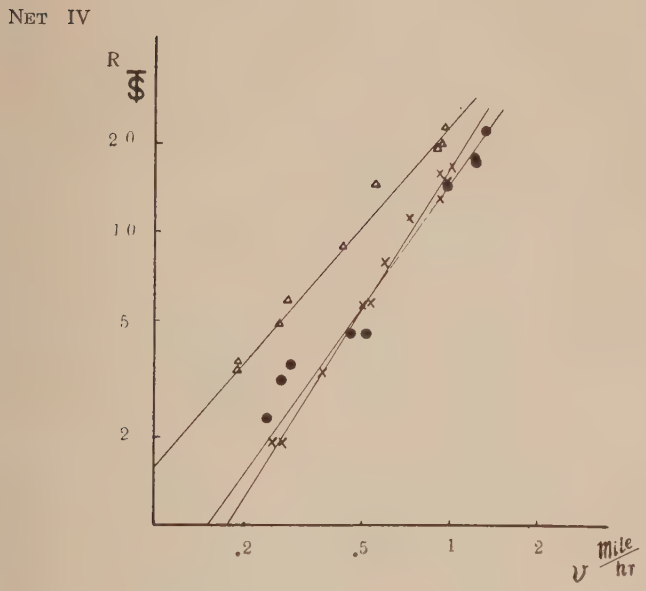


Fig. 6. Showing relation between R and v. (Net III, IV)



However, any simple numerical dependence of n on the degree of net deformation such as asserted by Dr. H. MIYAMOTO[7] does not seem prevailing among our present data. The reason for this variance will be inquired in future.

Acknowledgements

The present work has been performed in virtue of the assistance given throughout our exercise by a number of fishermen.

The author is indebted to Prof. M. TAUTI who gave many valuable critical advices in conducting the plan of the work and in preparing the manuscript for its publishment as well. Many thanks are also due to Dr. H. Hirano for his offering generous aids in finishing this paper.

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Table 1. Details of net.

	Name	Mark	Full scale			Model		
			Material	Diameter	Mesh size	Material	Diameter	Mesh size
			Manila	mm	cm		mm	cm
Net I	Hako Uodori (landing-net)	a	hemp	2.22	2.37	Silk	.27	.32
	〃	b	〃	〃	9.09	〃	〃	1.09
	〃	c	〃	〃	7.27	〃	〃	.87
	〃	d	〃	〃	5.45	〃	〃	.65
	〃	e	〃	〃	10.91	〃	〃	1.31
	〃	f	〃	〃	14.55	〃	〃	1.75
	〃	g	〃	〃	18.18	〃	〃	2.18
	〃	h	〃	〃	12.73	〃	〃	1.42
	Nobori (funnel net)	i	〃	〃	〃	〃	〃	〃
	〃	j	〃	〃	14.55	〃	〃	1.75
	〃	k	〃	〃	18.18	〃	〃	2.18
	Undoba	l	〃	〃	〃	〃	〃	〃
Net II	Hako Uodori (landing-net)	a	〃	〃	3.03	〃	.25	.34
	〃	b	〃	〃	4.55	〃	〃	.51
	〃	c	〃	〃	6.06	〃	〃	.69
	〃	d	〃	〃	7.58	〃	〃	.86
	〃	e	〃	〃	9.09	〃	〃	1.03
	〃	f	〃	〃	12.12	〃	〃	1.37
	〃	g	〃	2.85	〃	〃	.30	〃
	〃	h	〃	2.53	〃	〃	.29	〃
	〃	i	〃	2.22	15.15	〃	.25	1.71
	Nobori (funnel net)	j	〃	〃	12.12	〃	〃	1.37
	〃	k	〃	2.53	15.15	〃	.29	1.71
	Undoba	l	〃	2.22	〃	〃	.25	〃
Net III	Hako Uodori (landing-net)	a	〃	〃	3.64	〃	.27	.44
	〃	b	〃	〃	6.06	〃	〃	.73
	〃	c	〃	〃	7.58	〃	〃	.91
	〃	d	〃	〃	9.09	〃	〃	1.09
	〃	e	〃	〃	10.61	〃	〃	1.27
	〃	f	〃	〃	15.15	〃	〃	1.82
	〃	g	〃	〃	12.12	〃	〃	1.41
	〃	h	〃	2.53	〃	〃	.32	〃
	Nobori (funnel net)	i	〃	2.85	〃	〃	.38	〃
	〃	j	〃	〃	18.18	〃	〃	2.18
	Undoba	k	〃	2.22	〃	〃	.27	〃
	〃	l	〃	2.53	〃	〃	.32	〃
Net IV	Hako Uodori (landing-net)	a	〃	2.22	3.03	〃	.27	.37
	〃	b	〃	〃	4.52	〃	〃	.55
	〃	c	〃	〃	7.58	〃	〃	.92
	〃	d	〃	〃	6.06	〃	〃	.74
	〃	e	〃	〃	4.52	〃	〃	.55
	〃	f	〃	〃	9.09	〃	〃	1.10
	〃	g	〃	〃	10.60	〃	〃	1.30
	〃	h	〃	〃	12.12	〃	〃	1.45
	〃	i	〃	〃	13.62	〃	〃	1.63
	〃	j	〃	〃	15.14	〃	〃	1.82
	〃	k	〃	2.85	9.09	〃	.35	1.10
	〃	l	〃	2.54	〃	〃	.31	〃
	〃	m	〃	2.22	6.06	〃	.27	.74
	Nobori (funnel net)	n	〃	〃	13.62	〃	〃	1.63
	〃	o	〃	2.85	15.15	〃	.35	1.82
	Undoba	p	〃	2.22	〃	〃	.27	〃

Table 2. Details of ropes.

	Name	Mark	Full scale		Model	
			Material	Diameter	Material	Diameter
				mm		mm
Net I	Gawa-zuna (float-line)		Wire	24.0	Cotton	1.22
	Jinbari- 〃	n	Manila hemp	36.0	Silk	.43
	Dobari- 〃	o	〃	〃	〃	〃
		p	〃	〃	〃	〃
	Uwabari- 〃	q	〃	〃	〃	〃
	Sokobari- 〃	r	〃	〃	〃	〃
	Furasi- 〃	s	〃	〃	〃	〃
	Dohyo-zuna (sand-bag line)	t	〃	〃	〃	〃
	〃 〃	u	〃	〃	〃	〃
	〃 〃	v	〃	26.0	〃	.36
Net II	Gawa-zuna (float-line)		Wire	24.0	Cotton	1.18
	Dobari- 〃	n	Manila hemp	30.3	Silk	.45
		o	〃	〃	〃	〃
	Jinbari- 〃	p	Wire	24.0	〃	1.18
	Nobori- 〃	q	Manila hemp	〃	〃	.36
	Hari- 〃	r	〃	〃	〃	〃
	Dohyo-zuna (sand-bag line)	s	〃	27.3	〃	.41
	〃 〃	t	〃	24.0	〃	.36
Net III	Gawa-zuna (float-line)		Wire	24.0	Cotton	1.22
		q	Manila hemp	36.0	Silk	.52
		r	〃	21.0	〃	.32
	Dobari- 〃	s	〃	〃	〃	〃
		t	Wire	15.0	Cotton	.77
	Uwabari- 〃	u	Manila hemp	24.0	Silk	.36
	Sokobari- 〃	v	〃	21.0	〃	.32
		w	〃	24.0	〃	.36
		x	〃	〃	〃	〃
	Jinbari- 〃	y	〃	36.0	〃	.52
	Dohyo-zuna (sand-bag line)	A	Manila hemp	24.0	Silk	.36
	〃 〃	B	〃	36.0	〃	.52
	〃 〃	C	〃	〃	〃	〃
	〃 〃	D	Wire	24.0	Cotton	1.22
	〃 〃	E	Manila hemp	〃	Silk	.36
	〃 〃	F	Wire	〃	Cotton	1.22
Net IV	Gawa-zuna (float-line)		Wire	24.0	Cotton	1.27
	Hari 〃		Manila hemp	36.0	Silk	.54
	Dohyo-zuna (sand-bag line)	s	〃	〃	〃	〃
	〃 〃	t	〃	30.0	〃	.46
	〃 〃	u	Wire	24.0	Cotton	1.27
	〃 〃	v	Manila hemp	12.0	Silk	.18

Table 3. Details of float.

Name		Mark	Full scale				Model	
			Material	Length of bamboo	No. of bamboo	Buoyancy	Material	Buoyancy
				m		kg		g
Net I	Dai-aba	w	Bamboo	9.1	83	4.350	Cork	1.26
	Yabiki- 〃	x	〃	〃	65	3.420	〃	0.99
		y	〃	〃	60	3.150	〃	0.92
	Haguchi- 〃	A	〃	〃	1	87	〃	0.02
	Shoji- 〃	B	〃	7.6	22	797×6	〃	1.38
	Gawa- 〃	C	〃	〃	13	473×46	〃	6.38
	Yaku- 〃	D	〃	〃	22	797×4	〃	0.93
	Nobori- 〃	E	〃	4.6	2	43×6	〃	0.08
	Saruko- 〃	F	〃	3.8	11	198×19	〃	1.09
		G	〃	7.6	22	797×4	〃	0.93
Net II	Dai-aba	u	〃	9.1	27	1.852	〃	0.51
	Yabiki- 〃	u	〃	〃	〃	1.852×2	〃	1.02
	Gawa- 〃	v	〃	8.3	5	314×52	〃	4.50
	Shoji- 〃	w	〃	3.6	3	86×6	〃	0.14
	Tuzumi- 〃	x	〃	7.6	4	228×6	〃	0.38
	Nobori- 〃	y	〃	4.5	1	34×9	〃	0.09
	Saruko- 〃	z	〃	3.6	8	229×23	〃	1.45
	Yaku- 〃	z_2	〃	7.6	20	700×4	〃	0.77
Net III	Dai-aba	I	〃	7.6	95	4.170	〃	1.21
	Yabiki- 〃	J	〃	〃	〃	〃	〃	〃
	Gawa- 〃	K	〃	〃	7	308×51	〃	4.55
	Saruko- 〃	L	〃	3.8	13	210×31	〃	1.88
	Shoji- 〃	M	〃	7.6	15	660×4	〃	0.77
	Yaku- 〃	N	〃	〃	30	1320×8	〃	3.06
	Nobori- 〃	O	〃	〃	1	44×6	〃	0.08
Net IV	Dai-aba	w	〃	9.1	90	4.730	〃	1.37
	Yabiki- 〃	x	〃	〃	75	3.940×2	〃	2.36
	Yaku- 〃	y	〃	7.6	17	750×4	〃	0.87
	Nobori- 〃	z	〃	〃	1	44×8	〃	0.10
	Tuzumi- 〃	A	〃	〃	11	485×6	〃	0.85
	Gawa- 〃	B	〃	6.8	10	393×50	〃	5.70
	Shoji- 〃	C	〃	〃	16	630×5	〃	0.92
	Saruko- 〃	D	〃	3.8	8	129×44	〃	1.64

Table 4. Details of sand bags.

	Name	Mark	Full scale		Model	
			Material	Weight	Material	Weight
				kg		g
Net I	Dai-dohyo (sand-bag)	H	Gravel	6370×13	Lead	16.62
	Yabiki- 〃	I	〃	6370×14	〃	17.92
	Gawa- 〃	J	〃	6370×12	〃	15.35
	〃 〃	K	〃	5076×19	〃	19.38
	〃 〃	L	〃	3185×47	〃	30.20
	Undoba-iwazuna (lead-line)		Wire rope	384	〃	0.12
	Nobori- 〃		〃	69	〃	0.02
Net II	Dai-dohyo (sand-bag)	A	Gravel	12.760×8	〃	28.60
	Yabiki- 〃	B	〃	6.380×14	〃	25.10
	Gawa- 〃	C	〃	6.380×8	〃	14.30
	〃 〃	D	〃	3.190×52	〃	46.50
	Undoba-iwazuna (lead-line)		Chain	514	〃	0.14
	Nobori- 〃		Chain Lead	340	〃	0.09
Net III	Dai-dohyo (sand-bag)	P	Gravel	2.440×24	〃	17.56
	Yabiki- 〃	Q		2.440×23	〃	16.83
	Gawa- 〃	R		2.440×82	〃	59.90
	Undoba-iwazuna (lead-line)		Wire rope	320	〃	0.09
	Nobori- 〃		Lead	76	〃	0.02
Net IV	Dai-dohyo (sand-bag)	F	Gravel	2.880×13	〃	10.96
	Gawa- 〃	G	〃	2.880×44	〃	37.10
	〃 〃	H	〃	5.630×11	〃	18.55
	Yabiki- 〃	I	〃	2.815×14	〃	10.92
	Undoba-iwazuna (lead-line)		Wire rope	570	〃	0.17
	Nobori- 〃		Lead	656	〃	0.19

Table 5. The events effected by various currents on the net.

Current direction	Current Velocity (mile/hour)			
	Submargence of Dai-aba	Submergence of Hako-ami (or Undoba) floats	Blowing-up of net foot	Sand bags dragging
A	.25～.50	.33～.72	.17～.50	.72
B	.33～.50	.50～.72	.17～.50	.72～1.00
C	.33	.33	.17～.33	
D	.20～.25	.25～.33	.14～.33	.50
E	.20～.25	.25～.50	.16～.33	.72

Table 6. The current velocity on the actual fishing ground.

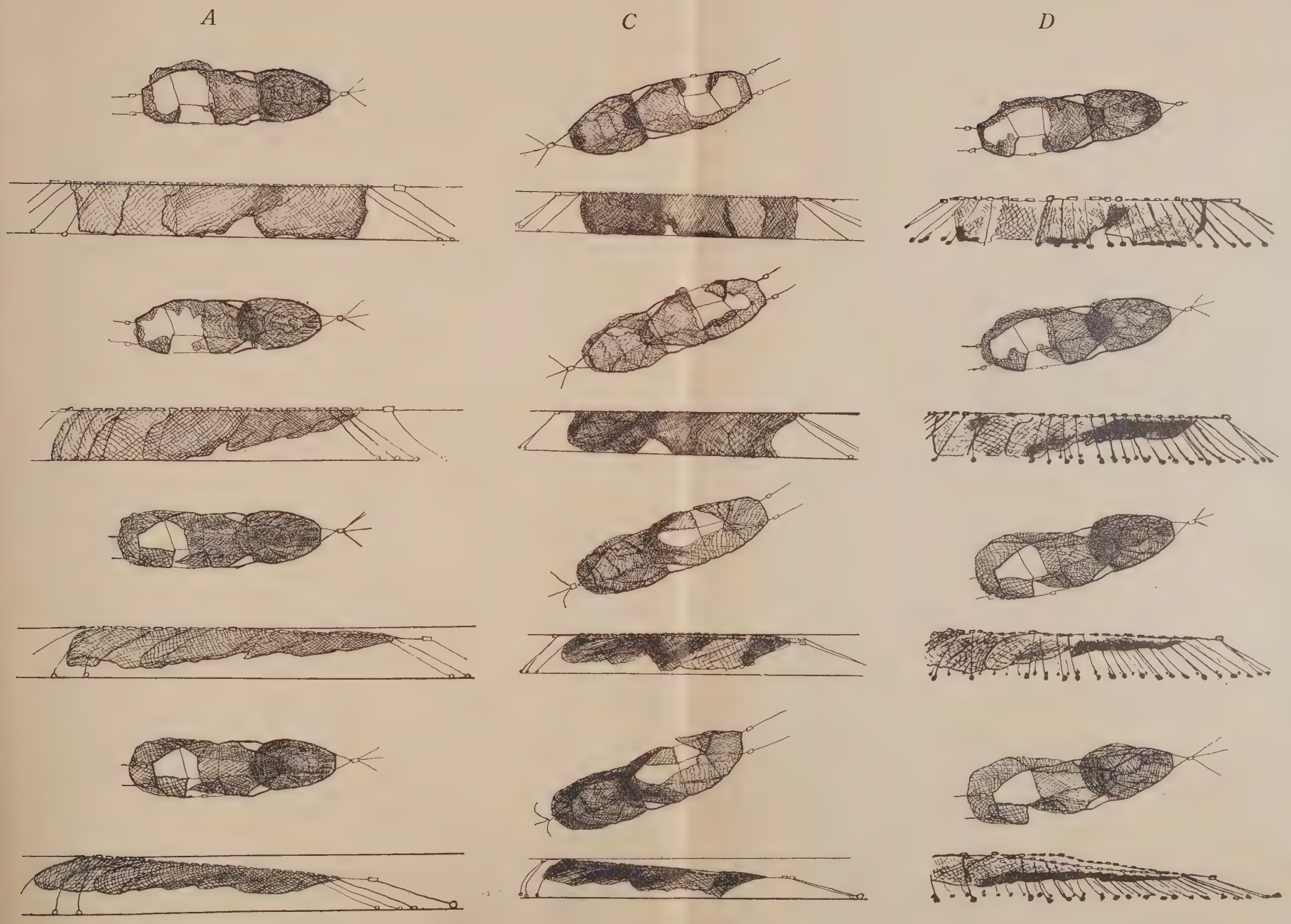
Net	Current Velocity (mile/hour)	
	Average	Allowable limit
I	very slow	.1
II	.2	.7
III	.2	.6
IV	.2	.5

Table 7. The caluculated values of k and n.

Net	Current direction	k	n
III	*A*	25.2	1.33
	B	19.2	1.50
	D	28.0	1.64
IV	*A*	16.7	1.63
	B	14.8	1.45
	E	23.5	1.17

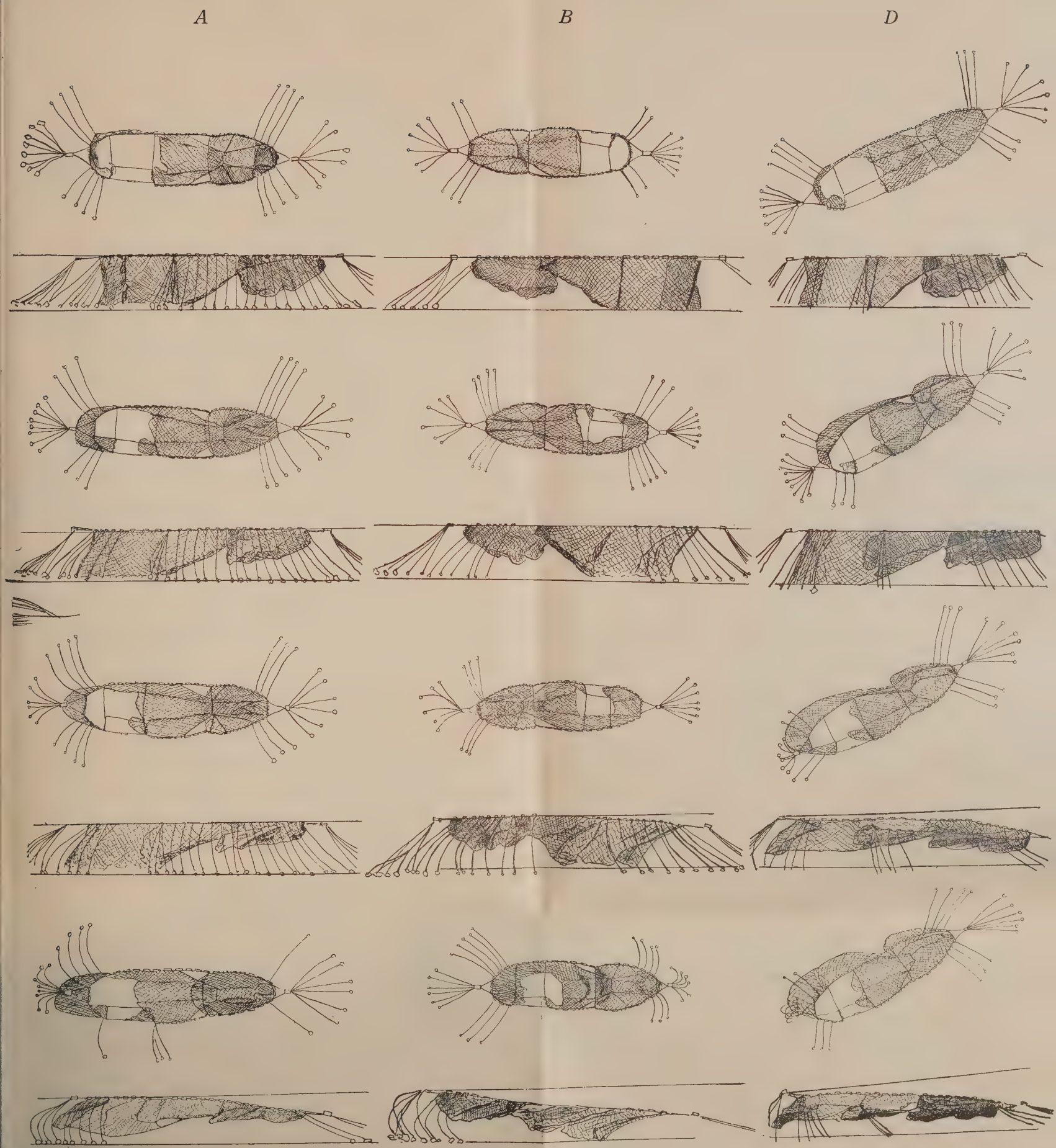
Fig. 4. Deformation of Nets I–IV. The Alphabets show the current direction and the numerals the current velocity, (mile/hour).

NET I

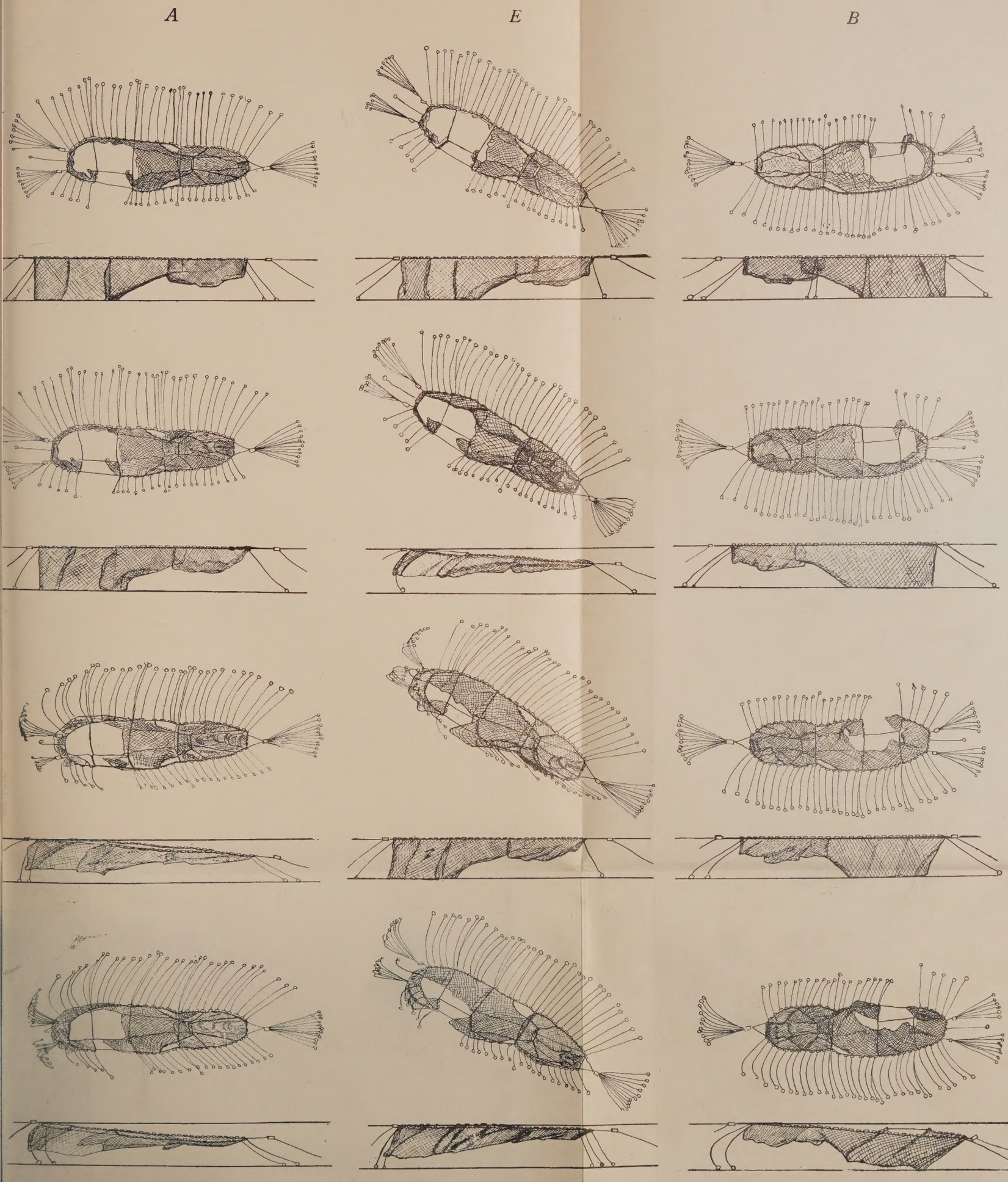


A
E
B

ET III



ET IV



NET I